The Determination of Trace Elements in Biological Systems by Activation Analysis.

by

Robert Alan Howie

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Summary of Thesis

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An introductory section defines the scope of the study, in terms of the elements to be determined, and a review of the techniques available, especially thermal neutron activation analysis. A discussion of the activation analysis technique is presented, including not only the relationship of this technique to the more usual analytical techniques, but also a synopsis of its application by previous workers in several fields.

New thermal neutron activation analysis methods are described for the estimation of antimony, iodine, and copper, zinc and cadmium in combination. All of these methods involve radiochemical separations, and their discussion illustrates the principles of this type of work.

Results of the application of the activation analysis technique to several problems are discussed. In medical research trace element analysis is of considerable significance. The estimation of copper by activation analysis, in studies on Wilson's disease provides results in agreement with those previously obtained by other analytical techniques. This study and other similar work on zinc and manganese demonstrate the potential value of thermal neutron activation analysis in the field of clinical medicine. The specificity of the activation technique is illustrated by its use as an alternative to the mass spectrometer in

stable tracer studies on the metabolism of calcium and strontium.

In the field of toxicology the method finds application in the present study in the investigation of the distribution of arsenic and mercury in those exposed to these elements. The sensitivity of the technique permits analyses which are impossible by any other analytical technique. Preliminary studies demonstrate the potential of the technique applied to forensic science problems. Proof of identity and the demonstration of the presence of gun-shot residues are both suitable for investigation by the activation analysis technique. Both problems have attendant difficulties which still remain to be overcome.

The routine application of the technique for the estimation of copper, arsenic, antimony, and mercury, provides a mass of data for the normal trace element content of human tissue similar to that obtained by other workers using x-ray excitation techniques. Similar methods are applied to such problems as trace element content of neoplastic tissue, as opposed to surrounding normal tissue in cases of lung cancer.

The present work describes the methodology of thermal neutron activation analysis applicable to biological systems. The applications described give some indication of the scope of the technique.

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(A) INTRODUCTION:

A.1. The Trace Elements:-

In discussing the possible elemental content of living organisms, it is reasonable to assume that such systems may contain each of the elements which are to be found in the material of the earth's crust. (54) The total number of elements to be found in the earth's crust is ninety, i.e. all the elements with atomic numbers (Z) between 1 and 92, with the exception of technetium (Z = 43), and promethium (Z = 61) which have been made artificially, as have the elements with atomic numbers greater than 92. The absence of these elements from the earth, and hence from living matter is ascribed to their nuclear instability. That is, they may have been present when the earth was first formed, but in the time that has elapsed since then, they will have been transmuted by the natural decay of radioactive materials.

Not all of the elements which might be found in living matter are trace elements. The bulk of such material is organic in nature, consisting of carbon, oxygen, hydrogen, and nitrogen. In addition, in man and the mammals, large amounts of phosphorus, potassium, calcium, magnesium, sulphur and sodium, are present. The elements in these two groups are classed as macro elements (82, 123). In the case of plants sodium is not usually a major constituent. The remaining eighty elements (eight-one in the case of plants) are delegated to the group of trace elements. Within this broad group a further sub-group is described, the essential trace elements (82, 123). For man and the mammals the elements in

this group are, iron, the most abundant member, which might possibly be classed as a macro element (82), chlorine, copper, cobalt, iodine, manganese, molybdenum and zinc. These elements are differentiated from the other trace elements because it has been shown that they are essential to the biochemical processes which distinguish living matter from its inanimate surroundings. The essential nature of these elements is due to their functional association with the biological catalysts or enzyme systems which accomplish the metabolic processes of a plant or animal, and is demonstrated by the failure of these processes in the absence of the trace element. It is evident from the close connection between the essential trace elements and life processes that determination of the essential trace elements is of great diagnostic importance in the examination of disease states. More important, once the role of, for example, cobalt in the form of vitamin B_{12}, or selenium as vitamin E (123, chap. VIII) is understood the diseases arising from deficiency of these elements may be treated successfully with the appropriate addition to the diet.

The remaining trace elements, seventy-one in number, including selenium, whose essential nature is still in doubt, are not classed as essential, merely because their essential nature has not been unequivocably demonstrated. Failure to show the presence of any of these in a particular biological system does not preclude a possibility of that element having a role to play, but merely points to the inadequacy of the analytical technique used. At the present time, in the absence of other evidence, if trace elements other than the essential trace elements are found to be present, their presence

is ascribed to accidental inclusion, or contamination.

Therefore, the elemental content of a biological system can be divided into three groups. First there are the major constituents, forming the organic matrix of the system. Second there are the essential trace elements, which although present in small amounts exert a profound influence on the function of the system. Lastly there are the other trace elements which do not appear to have any function in the system, and are merely distributed through it.

A.2. The Estimation of Trace Elements:-

Since the trace elements are present in biological samples in such small amounts, not more than say 0.005%, the iron content (4 g) of a standard 70 Kg man (82), and more often several orders of magnitude lower than this, it is obvious that only the more sensitive analytical methods are applicable. This excludes gravimetric analysis entirely, and only the most sensitive titrimetric methods are used, and even then inconveniently large samples are often required. The remaining methods of classical chemistry, polarography, colorimetry, and flame photometry in its various forms, have all been used in the estimation of the trace element content of biological material. The most widely used method of these three is colorimetry. At trace element level the usual chemical manipulations are difficult, due to the ease with which relatively significant amounts of material can be lost by absorption on container surfaces, co-precipitation with unwanted material, and sometimes the failure of the usual chemical reactions. For example, if only a very small amount of an element is present it may be impossible to precipitate it as its solubility product

cannot be exceeded. It is partly considerations of this kind which limit the sensitivity of the classical method of analysis.

In colorimetric and flame photometric methods, the sensitivity is restricted to a number of atoms of the trace elements which will absorb or emit detectable amount of energy. Now since the process giving rise to the emission or absorption of energy in these methods is one involving electrons, either in the element itself or in the orbitals of a molecular association of which it is a part, the energy change for each trace element atom detected is only a few electron volts. The best way to attempt to achieve the utmost sensitivity, and the ideal case where individual atoms can be detected, might lie in arranging that the energy change associated with the detection of a single atom is of the order of a few kilo-electron-volts.

This ideal cannot be achieved if the excitation or relaxation effect is confined to orbital electrons. Even in x-ray emission spectroscopy, or x-ray fluorescent analysis, (54) where K and L electron shell transitions are produced by x-ray bombardment, and the resultant fluorescent radiation is resolved by diffraction and counted in a detector similar to those used in radiochemical techniques, the technique is limited in sensitivity to the estimation of any particular element at a level of about 0.1 p.p.m. due to the statistical requirements of the detection equipment. The x-ray technique is similar to activation analysis in several ways, such as its application to a wide range of elements, its ability to permit non-destructive analysis, and the nature of the excitation process which renders the technique a method of elemental analysis, taking no account of the chemical state of the element determined.

The desired energy is, however, well within the range of the energy changes involved in transitions within the nucleus of a single atom. Thus the main hope of achieving the ideal sensitivity must lie in examining the processes involved in nuclear excitation or radioactivation, or the resulting radioactive decay. This is the principle on which activation analysis is based, but the ideal sensitivity cannot be achieved, because although it is possible to detect the product of a single decaying nucleus, the statistical nature of the decay process renders impossible the estimation, and identification of a single decaying nucleus. Further, the initial activation process is also statistical in nature. The result of these two effects is that, even using activation analysis, the best that can be achieved as regards sensitivity is the detection, in favourable circumstances, of a thousand million or so atoms of a particular element. For an element of atomic weight 100, this might represent a limit of detection of about 2×10^{-13} g.

It would appear from this discussion that activation analysis offers the best hope for extending analytical sensitivity for the estimation of trace elements, as it utilises nuclear transitions involving a vastly greater amount of energy per atom than the electroni changes associated with the methods of classical chemistry.

A.3. Activation Analysis:-

The technique of activation analysis was an early by-product of the work carried out to investigate the structure of atomic nuclei, and if possible to harness the power stored in them. The success of

the early work is amply demonstrated by the modern use of nuclear power plant for commercial production of electricity. Activation analysis, too, has progressed from its early beginnings, and has found wide application as a sensitive and accurate technique for the analysis of many materials. Since the earliest applications of the technique by Hevesy and Levi (67) it has been employed in such diverse fields as metallurgy, medicine, public health and forensic science. Two texts are available, by Bowen and Gibbons (25) and by Lyons (88), each giving some indication of the scope of the method, as well as complete manipulative instructions so that it could be used in any laboratory, provided the necessary equipment is available. Guinn and Smith and Lenihan (140, 59) have contributed chapters to Curry's 'Methods of Forensic Science' where some practical details are given, and possible applications are discussed.

The volume of literature presently appearing on activation analysis is indicated by reviews and bibliographies such as those of Leddicotte, Bock-Werthman, and the United Kingdom Atomic Energy Authority (81, 13, 157). Despite the large volume of literature on activation analysis, relatively little has been published on its application to medical science. As a result, the bulk of the new material appears in the literature of pure and applied chemistry, rather than that of chemistry in medical science.

The principles of activation analysis are quite simple. Essentially the sample for analysis is activated by bombarding it with sub-atomic particles, whole nuclei, or even gamma radiation. As a result the nuclei of the constituent atoms of the sample undergo

rearrangements, and become radioactive. The remainder of the technique involves separating the activity due to the element to be measured, from the other induced activities and evaluating it quantitatively. The separation of the activities may be accomplished instrumentally, retaining the activated sample intact, or alternatively radiochemistry may be employed either to isolate the desired activity completely free from contamination, or at least freed from the bulk of interfering activity. The use of a complete radiochemical separation usually gives the greatest possible sensitivity, but the purely instrumental approach, or the combined chemical and instrumental technique are of value when it is desired to determine several elements simultaneously.

To evaluate quantitatively the process of activation, it is necessary to consider first the nature of radioactive decay. For an isolated radioactive nucleus it is only possible to say that it has a probability (λ) of decaying within a given time. It follows that the number of nuclei which do in fact decay if there are N present in a sample is given by:- $dN/dt = -\lambda N$, where dN/dt is the rate of decay ..... (a). On integration this gives the number of atoms left after decay for a time t as

$N = N_o e^{-\lambda t}$, where N_o is the number of radioactive atoms present at time t = 0.

For any target nuclide, the rate of activation (P) depends only on the number of activating particles available, usually expressed as a flux (ϕ) particles/cm^2/sec, the number of target atoms present in the sample, (N_1) and the probability of any one of these target

atoms undergoing the desired nuclear reaction. This last is expressed by assigning to the target atom a cross-section for the reaction, expressed in cm^2, although to conform with the actual dimensions of atomic nuclei, the cross section (σ) is normally tabulated in barns, where 1 barn is 10^{-24} cm^2.

Now the rate of production of activity from the target is given by

$$P = N_1 . \phi . \sigma \quad \ldots\ldots\ldots\ldots \quad (b)$$

As soon as radioactive atoms are formed, they can decay, subject to the decay law set out in (a) above, so that the nett rate of production of active atoms is given by: - $dN_x/dt = P - \lambda N_1$ .... (c)

Transposition and integration of (c) gives the number of active atoms present after activation for a time t as:-

$$N_x = (P/\lambda).(1 - e^{-\lambda t}) \quad \ldots\ldots\ldots\ldots (d)$$

Finally the activity detectable, which is the rate of decay of the active atoms, is given by multiplying (d) by the decay constant of the active species, so that

$$\text{Activity} = dN_x/dt = P(1 - e^{-\lambda t}) = N_1 . \phi . \sigma (1 - e^{-\lambda t}) \quad \ldots\ldots \quad (e)$$

A plot of the induced activity in any arbitrary units, against the time of irradiation, expressed in units of the half life of the induced activity gives a curve of the form shown in fig. A.1. The main features of this growth curve are its assymptotic approach to a saturation activity, which corresponds to the situation where the rate of decay of the active species is equal to its rate of formation, and its approximation to linearity for irradiation times up to one half life of the product activity. This last indicates that it is

rarely useful to activate for more than one half life of the product activity.

The relationship (e) above is more useful for the estimation of sensitivities or the activity produced by a given amount of target material by a particular reaction, if N_1 is replaced $(w \cdot N_{Av}/ A) \times B$, where w is the weight of target element, N_{Av} is Avogadro's number, A is the atomic weight of the target element, and B is the fractional abundance of the particular isotope of the target element which is producing the desired activity. Usually the total induced activity is a combination of the activities induced by several reactions occurring simultaneously. One other factor which must be considered is that detectors for radioactive assay vary in efficiency, and allowance must be made for this, in converting the activity produced (in disintegrations per second, or curies, where 1 curie is 3.7×10^{10} disintegrations per second) into counts per second as actually registered on the detector system used. For calculations of sensitivity in practical applications (e) now is used in the form:-

$$\text{counts/sec.} = \frac{E.w.N_{Av}.\ B.\phi.\sigma.}{A}\ (1 - e^{-\lambda t}) \quad \ldots\ldots\ldots\ldots (f),$$

where all the symbols have the same meaning as before, and E is the detector efficiency. A useful device, based on this type of relationship, is the isotope calculator of Eastwood (42). The device is essentially a specialised form of slide rule, which permits the calculation of specific activities in curies/gm, for an element of given atomic weight (A), with a given cross section (σ), on irradiation for a given time (t) at a stated flux of activating particles (ϕ). The decay constant (λ) of the product must also be

known. Some tabulations of nuclear constants, e.g. Allen et al (2) simplify the situation for the elements with several isotopes, by quoting not only the cross section for the desired reaction of the participating isotope, but also a cross section for the activation of the natural element, which of course takes account of the isotopic abundance of the target nuclide.

The bombarding particles which may be used for activation analysis include fast and thermal neutrons, charged particles such as protons or even larger atomic nuclei, or even high energy gamma rays. The most promising of these for trace element analysis in biological systems are thermal neutrons. First, such particles are readily and cheaply available in atomic reactors at fluxes great enough to give high sensitivity for activation analysis. Second, most of the elements react fairly readily with thermal neutrons, forming neutron rich products. The reaction involved (n,γ) tends to be more easily accomplished than the (n,p), or (n,α) reactions which are typical in the use of fast neutrons, and these in turn are more efficient reactions than those involving charged particles or gamma rays as the activating species. Third, the elements which are not activated by thermal neutrons are those at the beginning and end of the periodic table. Thus the matrix material of a biological system, consisting of hydrogen, carbon, oxygen, nitrogen and possibly sulphur, produces scarcely any detectable activity. Even sulphur, which will become radioactive on pile irradiation does so, not by neutron capture, but by an (n,p) reaction, not due to thermal neutrons but to the accompanying fast neutron flux, in the spectrum of neutron energies

produced in the fission process on which the pile runs. At the other end of the periodic table, there are the heavy elements, which do react with thermal neutrons, but since the reaction produces a complex mixture of fission fragments, which are difficult to assign, thermal neutron activation analysis will rarely serve for their estimation. Although it is theoretically possible to determine the amount of a particular element present in a sample, simply by consideration of the actifity detected, and the use of a formula such as (f) above, in practice the nuclear constants required for such a calculation, and errors in the estimation of detector efficiency render this approach inaccurate. As a result the method is always best applied as a method of comparative analysis. Standard samples containing accurately known amounts of the elements to be estimated are irradiated along with the sample, and processed in an identical way. Analysis is achieved by comparison of the activities in standard and sample.

The activation process is not selective with regard to the activities produced. Some restriction on the activities present in the activated sample may be imposed either by careful control of the time for which irradiation is carried out, or more important, by delaying the activity estimation until the shorter lived interfering activities have decayed. In general however, it will be necessary to pick out the activity arising from the activation of the desired element from a complex mixture. This may be done by gamma spectroscopy (Crouthamel, 39) which depends on the relatively good separation in the energies of the gamma rays emitted in the decay of most but not all radioactive species. This technique is also used in non-destructive

analyses. The technique which permits estimations of the greatest sensitivity involves the radiochemical separation of the desired activity, usually to such an extent that any activity detected may be ascribed to the element to be determined. Intelligent combination of the two approaches results in activation analysis methods which, though not of the greatest possible sensitivity, permit the rapid estimation of very small amounts of the element concerned, in a large number of samples, with adequate precision.

Since activation analysis is aimed at the estimation of very small amounts of particular elements, it would appear that the associated chemical manipulation would be very demanding. Apart from the requirements of adequate radiochemical purity this is not so. Chemical separations are accomplished by the use of carrier techniques. Convenient amounts of inactive material, usually the same element as the activity to be isolated, is added, and if the carrier is chemically equilibrated with the active material from the sample, then the activity will be distributed through the separation along with the carrier. The efficiency of any chemical process used is readily obtained from the yield of carrier material which can be estimated in the normal way, by gravimetric analysis, or by colorimetry, or even in some instances by a simplified activation analysis technique (91, 71), involving only activation and counting. Since carriers are used, the chemistry involved can be carried out on any convenient scale, so that virtually any method of classical analysis can be adapted to activation analysis. Further, since the yield of the chemical processing can be readily determined, processes may be used in activation analysis which are not usually considered sufficiently efficient for use in classical methods.

The usual techniques of classical chemistry including precipitation, solvent extraction, ion exchange, volatilisation and paper chromatography find application in activation analysis. Since the time available for the separation depends on the half life of the activity to be estimated the separations used are often designed for speed of operation rather than the highest possible yield. Normally exactly the same amount of inactive carrier is added to both sample and standard, and the activities and weights separated from sample and standard are determined. The content of the sought for element in the sample is given by

$$\%\text{age content} = \frac{\text{wt. element in std.}}{\text{wt. of sample}} \times \frac{\text{cts. sample}}{\text{cts. std.}} \times \frac{\text{wt. recd. from std}}{\text{wt. recd. from sample}} \times 100$$

An elegant refinement developed by Ruzicka and Stary (118) eliminates the need to actually measure the recoveries from the chemical processing. Sample and standard both have the same amount of carrier added to them, and equality of recovery is guaranteed by the use of less than the stoichiometric amount of reagent for the final separation step. Thus in the precipitation of iron by this means, only sufficient hydroxide would be added to both sample and standard to precipitate 80% of the added carrier. Exactly the same amount of reagent must be used for sample and standard.

Another technique which is suited to rapid radiochemical separations utilises the equilibria established between precipitates and the residual constituent ions in solution or between a complex in a liquid phase immiscible with water, and the original aqueous solution. Meinke and his co-workers have developed and utilised separations of this kind for several elements (110). A related technique, limited in application to the metallic elements is that of amalgam exchange (41). This utilises the equilibrium between a metal and an aqueous solution

into which it dips, as in electrochemical half cells. The function of the amalgam is mainly to accelerate the equilibrium process by the fine division of the metal possible in its amalgamated form, permitting the minimum amount of alloyed metal to have the maximum effect. The method here, as in the method quoted above, is merely to shake the amalgam with a solution of the desired activity in a suitable valency state, and the activity distributes itself between the dissolved ions and the very much larger number in the amalgam. The concentration of the element in the amalgam is so much higher than in the solution that the extraction of the activity of the desired element into the amalgam is virtually complete.

The efficiency of a radiochemical separation of whatever sort is determined from the nuclear characteristics of the isolated material, such as half life, gamma energies, and in some cases beta energies. These last are not so valuable in the estimation of radiochemical purity as the other methods, but can be estimated fairly easily from beta absorption studies. The ideal case is where no activities other than the desired activity can be detected. Then any activity which is detected may be ascribed to the element for which the analysis is made. This situation reduces the lower limit of sensitivity very greatly for a given set of irradiation conditions, as the limit on the sensitivity of the detector system is largely determined by the background count arising from cosmic radiation, and radioactivity in the counter itself, and its surroundings. More significantly the limit of detection is governed by the error in the background, in combination with the error in the count from the isolated material obtained in the presence of the

background activity. Detailed discussions of the statistical considerations involved in this as in any other problem on the accuracy and limits of the assay of radioactivity will be found not only in the activation analysis texts (25,88), but also in the standard radiochemistry texts such as that of Cook and Duncan (37).

The alternative to radiochemical separations of the kind described above is to attempt separation by evaluation of the energies of the particles emitted in the decay of the sample and if possible to assign them to specific activities. This can be done most effectively for gamma radiation, and the technique employed is that of gamma spectrometry. The equipment involved is well described in the general texts (25, 88, 37) and further accounts are available in books by Putman (109), Crouthamel (39), and in United Kingdom Atomic Energy Authority reports such as those by Salmon (120).

The necessary equipment can be presented in the form of a block diagram (fig. A.2.). The essential features are:-

i. the thallium activated sodium iodide crystal, whose function is to convert the incident gamma ray into a proportional amount of light,

ii. the phototube, which converts the incident light from the crystal into an equivalent number of electrons, - these are then amplified to give a detectable pulse by accelerating them down a series of dynodes contained in the photomultiplier, the amplification being determined by the high tension applied to the dynode chain by the E.H.T. unit,

iii. an amplifier system, usually operating in two stages,

iv. some form of analyser system, which can sort out the pulses from the amplifier system according to their size, which corresponds to the energy of the gamma ray which produced them initially,

v. some form of recording system, such as a scaler, or ratemeter, or the memory of a multi-channel analyser.

Although the principles of the technique are relatively simple, in practice it is complicated by the non-linear relationship between the final pulse from the amplifier system and the incident gamma ray. The deviation from linearity commences in the conversion of the gamma ray into light in the crystal. The way in which gamma rays interact with matter result in only some of the incident gamma rays undergoing conversion into light completely, (the photoelectric effect), and a proportion of them are scattered instead (Compton effect), and others again if of sufficient energy may produce positrons in the crystal with part of their energy, and some of this energy may escape detection when the positron is annihilated, resulting in the formation of escape peaks. The other major limitation to the detection system lies in the response of the photomultiplier. The gain of this device is very sensitive to changes in the high tension supplied to it, and even in the absence of detectable changes in high tension the range of amplification achieved results in gamma rays of a particular energy appearing as Gaussian peaks in the final spectrum.

A gamma spectrum of the form shown in figure A.3. is obtained by plotting the number of counts in a given time against the size of the pulse obtained. The curve shown is the typical spectrum of Cs^{137}, where the photopeak corresponding to the gamma energy of 0.637 MeV is

clearly seen, as is the Compton continuum, which contains counts obtained in events where the incident gamma ray was scattered in the sodium iodide crystal. The main difficulties in gamma spectroscopy lie in the identification of the significant photopeaks, their assignment to the correct activity, and the quantitative estimation of the amount of each gamma present, despite the presence of interference from the Compton continua of photopeaks of higher energy. In the case of non-destructive analysis, this can be an extremely difficult task, requiring the use of some such device as a spectrum stripper, which permits the sample spectrum to be taken to pieces bit by bit, or alternatively computer techniques may be amployed. The situation can often be simplified considerably, at the cost of destroying the sample, if radiochemistry of the simplest kind is employed.

It appears that the most promising method of activation analysis for the estimation of trace elements in biological systems is that where the initial activation process is carried out by thermal neutrons. The majority of the elements in the periodic table will react with these, and among the elements which are not activated are those forming the matrix of biological material. Although more recent workers have attempted analyses from the investigation of the prompt gamma radiation emitted in the activation process (56), the majority of the methods presently available are based on the separation of the mixture of induced activities. This is often done radiochemically, using either radiochemical separations, such as those published by the National Academy of Science in a series of booklets covering all the elements in the periodic table (101), or modified methods from classical chemistry.

The alternative, less sensitive, and less generally applicable technique is the gamma spectrometric method. This demands complex equipment, and considerable skill, both mathematical and technological, for its successful operation.

A.4. Advantages and Disadvantages of Activation Analysis:-

The principle claim for the adoption of thermal neutron activation analysis for the estimation of trace elements in biological systems is the great sensitivity of the method for most of the elements of interest. A valuable comparison between activation analysis methods, and the more common classical methods is reproduced, in a shortened form in table. A.1. The original was published by Meinke (93), For ease of comparison, the sensitivities for all methods are expressed in micrograms per ml, and it is assumed that if necessary there is 25 or 50 ml of solution at the quoted concentration.

For activation analysis, the sensitivity for the estimation of a given element depends on the activation reaction used, and the cross-section of the element for this reaction. In this instance discussion is limited to activation by thermal neutrons, and the cross-sections considered for each element will be those for the capture of thermal neutrons. The other major factors governing the activation process are the neutron flux, which is assumed to be 5×10^{11} neutrons/cm^2/sec, and the time of the irradiation, which as we have seen should be considered in terms of the half life of the product activity. For the purposes of the table irradiations are supposed to have been carried out for 30 days, or to saturation, whichever is the shorter period. The sensitivity of

the assay of the induced activity is set at the detection of 40 disintegrations per minute as the lower limit. An important consideration in using a nuclear reactor for irradiations of this kind is the space available for the sample, which of course limits the size of the latter. Accordingly it is assumed that 1 ml aliquots of the sample solution are activated.

For the methods of classical chemistry with which activation analysis is to be compared, the only comment that need be made is that the sensitivity for the colorimetric methods assumes the use of a 1 cm path length, and 1 cm^2 area of cell and solution interposed in the colorimeter light beam. This is of course quite consistent with the microgram per ml scale used for the other methods.

In table A.2. those elements with sensitivities for activation analysis of less than 0.1 μg are classified in groups according to sensitivity. First with sensitivities less than or equal to 10^{-3} μg/ml under the conditions stated above, there are the elements including manganese, which is known to be of biological importance, and vanadium. In the next group there are iodine, sodium, and copper, all three essential trace elements. The next group, with sensitivities in the range 10^{-1} to 10^{-2} μg/ml contains chlorine, cobalt, and zinc. Outwith these sensitivities are found the major constituents of biological material (possibly excepting calcium and rhenium), the rare gases and the elements with atomic numbers 84 to 91 and 1 to 10. These are not of interest in a study of trace elements. Again from table A.2. it is seen that 11 elements give sensitivities of 10^{-3} μg/ml or better, 29 give sensitivities of 10^{-2} μg/ml or better, and 51, or just more than half

of the elements in the periodic table, give sensitivities of 10^{-1} μg/ml or better. Table A.3. summarises those elements for which activation analysis sensitivities calculated on the assumptions stated above, are inferior to those of the classical chemical methods. Although the list is disappointingly long, the situation is in fact not quite as unfavourable towards activation analysis as it might appear, because the flux on which the calculations are based is in fact about half of that usually available, and when the chemistry involved in the various methods is taken into account, many elements are retrieved from the list in table A.3. with the principal exceptions of the blood electrolytes, and the alkaline earth metals. This does not mean that thermal neutron activation analysis for these elements is not worth investigation, as the technique may well have something to offer in an attempt to estimate several of them in combination in a single sample, or even in the rapidity with which a single element analysis can be achieved.

The extreme sensitivity of the activation analysis technique is also a factor which adds to its difficulty. The activation process, as has been shown, is relatively non-selective. Any material accidentally added to the sample before irradiation will be activated along with the material of the sample itself. The extreme sensitivity of the technique results in the situation that the amount of contamination which the method can tolerate is very slight indeed. Pre-irradiation contamination is kept to a minimum by handling material for analysis as little as possible (132). Further chemical treatment before irradiation is not advisable (94) unless contamination can be controlled. One technique which solves the problem is to make us of the fact that certain

samples such as seeds are sealed in natural containers, and can be unpacked from these only after irradiation. A similar technique can be applied to the estimation of the trace element content of the organs of small animals. The animal is irradiated whole, and the desired tissue dissected out afterwards. This type of manipulation may be rather difficult due to the amounts of radioactivity which may be encountered. The number of cases to which this approach can be applied is limited, and most often the best that can be done is to keep the handling of material to a minimum, and to try to use instruments in sample preparation which do not contain the elements which are to be determined. Polythene provides a suitably inert material for the storage of samples in most cases. Stainless steel scalpels and forceps can be used, but it is safer to use polythene forceps and spatulas, and knives are best made from silica.

Some workers have used pre-irradiation chemistry of various kinds to concentrate samples prior to analysis, by such means as ion exchange for sea water, or by dry ashing for samples of biological material (95). These are subject to contamination risks, and the dry ashing technique is even more hazardous due to the volatility of many elements, which although the effect may be slight in normal circumstances, becomes a major source of error in the low concentrations present if the ashing technique is necessary to achieve the analysis. Loss of the element for which the analysis is designed may occur even without the application of the extreme conditions involved in ashing the sample, as for example, the loss of selenium from blood to the surface of the stainless steel needle used to obtain the sample, described by

Wainerdi (161). This was overcome by the use of a polythene syringe.

Once the activation process has been achieved, the sample can be dealt with as necessary to separate the desired activity from the mixture of induced activities. This may be accomplished by physical means, with the advantage that the sample is preserved intact. More commonly it is necessary to isolate the desired activity by some chemical process. The activation analysis method has several advantages to offer at this stage over the more usual methods of trace element analysis. First, since the desired element in the sample has been labelled by the activation process, it is simple to distinguish portions of the same element which may now be accidentally added to the sample by the use of sub-standard reagents. In almost all colorimetric analyses it is accepted practice to allow for this effect by preparing a blank solution to represent the contribution of the reagent contamination to the trace element content of the sample as actually measured. This will not be required for an activation analysis method unless the sample is processed in some way before it is activated. Second, the addition of inactive carrier material permits the chemical operations involved in the separation to be carried out on any convenient scale. This eliminates the difficult micro-manipulations normally associated with trace element analysis. Further, since the yield of any chemistry employed may be readily estimated from the return on the amount of carrier added initially, the time consuming quantitative operations of the classical techniques is no longer essential. The carrier technique can only be successful if a deliberate attempt is made to render the added carrier and the active material from the sample homogeneous, and

chemically indistinguishable. This is achieved by a drastic oxidation or reduction step, whose purpose is to make the carrier pass through all the valence states and chemical forms which the product of the activation process may adopt.

One last advantage of the activation analysis is the possibility of estimating a single stable isotope of an element which normally exists in one or more stable isotopic forms. This specificity of analysis approaches that of the mass spectrometer. It is due of course to the fact that the activation process acts on the atomic nuclei, and the effects which renders an activated nucleus detectable are characteristic of that nucleus. This also means that activation analysis can only be a method of elemental analysis, because the configuration of the valence electrons, which determines the chemical state of a particular atom, has no effect on its properties with regard to activation. In addition the energy changes associated with the nuclear excitation process are so large relative to the energies of the orbital electrons, that a common consequence of activation is a change in valency of the activated atom, often accompanied by the rupture of a chemical bond. A common example of this is the Szilard-Chalmers effect, where the exposure of ethyl iodide to neutrons results in the formation of active iodine which can be extracted by shaking the activated material with water (150)

On the debit side the method suffers from the major disadvantage that it involves work with radio-isotopes. These are potentially dangerous, but although the conditions normally applied to any radio-chemical operation are enforced, activation analysis usually involves activity levels much lower than those encountered in other applications

of radiation. There is no question that it might not always be possible to obtain suitable irradiation facilities, but in Britain this difficulty is now readily surmounted, thanks to the United Kingdom Atomic Energy Authority Irradiation service using the reactor BEPO at Harwell, and the availability of the Scottish Universities Research Reactor at the National Engineering Laboratory site at East Kilbride. The other major requirement is the detector equipment. Geiger counting of the simplest kind is quite satisfactory for many analyses, but for the more refined techniques involving scintillation detectors, the equipment tends to be rather expensive. These are objections solely on the grounds of capital cost, and of no significance in a discussion of the limits of the technique.

More important to an assessment of the potential of the activation analysis technique are the errors to which this analysis method is peculiarly susceptible. These can be classified according to the phase of the method to which they apply. For example in the irradiation process there is the possibility of systematic error due to inhomogeneity of the bombarding flux. This may be a characteristic of the particle source used, or may be induced in the sample itself by the presence of strongly absorbing material, so that the flux present at the centre of the sample is not the same as that at its periphery, due to self-shielding (3). The former effect is dealt with by monitoring the flux at different positions in the irradiation container. The second effect is rarely encountered in analysis of biological materials. Certainly it will not occur in the thermal neutron irradiation of the samples themselves, where the trace elements are

dispersed in an inert matrix. It may be an error arising in the activation of the comparator standards, which are often relatively large amounts of strongly absorbing material. When deciding how much material to use in such a standard, it is usual practice to irradiate several solutions, of different concentrations, and to plot the specific activity obtained against the sample size. This should of course give a horizontal line (fig. A.4) in the absence of self-shielding (49) and from the deviation from this line the size of standard which gives an error due to self-shielding within acceptable limits may be determined (30). A further source of error in the activation process is the possibility of the production of the desired activity by a competing reaction from some other target nuclide (78,162). In irradiation with pile neutrons the thermal flux is invariably accompanied to some extent by neutrons of slightly higher energy. The more energetic neutrons can produce activity by (n,p) or (n,α) reactions which are the same as the product of a desired (n,γ) reaction. Consideration of this type of interference shows that it can only come from elements near to the element to be estimated in the periodic table. This is a primary interference, and again, from the nature of biological samples is rarely of significance in this type of material. Another type of competition which may occur is that of secondary reactions. In this case, the incident particles displace nuclei inside the sample, which then act as bombarding particles. For example, Gilmore and Hull (52) describe the formation of N^{13} in the irradiation of pure hydrocarbons with high energy (14 MeV) neutrons. The active product is formed by the capture of protons, which have been

'knocked on' by the incident neutron flux, by the carbon atoms of the sample. Both of these effects are said to be of first order, as they involve a single activation step. Second order interferences arise when either the desired activity is produced from the wrong target nuclide, or the desired nuclide itself is reduced in amount by a process involving a decay step, and a further activation step. This type of interference is by its very nature not very significant, and if the effect is to diminish the amount of product activity, this will operate equally on the samples and the standard in the comparator technique.

If chemistry is applied for the isolation of the desired activity prior to its assay, this process has its attendant difficulties. First there is the problem of equilibrating the added carrier with the activation product in the case of elements which can adopt two or more valence states in nature. Second, although the separations may be relatively simple since a yield determination is carried out, the decontamination factors which must be achieved are much higher than in the classical methods, because the activation process renders not only the desired element detectable in very small amounts, but also possible contaminants.

The assay of the isolated activity is also rather more complex than might be supposed. Any count obtained must be corrected for (i) the natural activity of the counter and its surroundings; the background count, (ii) the response time of the detection system; the dead time, and (iii) the decay of the sample activity as counting proceeds. All of these effects are common to any counting system, and

detailed information on them is obtainable from any radiochemistry text (25, 88, 37). In addition the statistical nature of the radioactive decay process, and of the counts obtained from it delineate the conditions under which counting is carried out. That is the allocation of available counting time to estimation of background or sample activity must be deliberately arranged to permit the estimation of the activity of the sample alone within the accuracy required for the analysis.

In non-destructive analysis, or multi-element analysis, using gamma spectrometry difficulties are encountered, first in identifying the peaks in the spectrum, and more important the activities producing them, and second in evaluating the contributions of the individual components to the total spectrum.

Thermal neutron activation analysis would appear to be an excellent method for the estimation of trace elements in biological systems. The simplicity of the principle involved, and its close links with classical chemical methods, and the great sensitivity attainable in many cases, all seem to be strong arguments for its application. Activation analysis is not the only application of radio-isotopes to analytical chemistry (80, 168), where radioactivity finds many applications as a means of labelling small amounts of material in a readily detectable form. For some elements activation analysis does not give good sensitivity because of the long half life of the product activity. In this case the sensitivity is improved by applying the related technique of isotope dilution (80, 118, 16), the inactive sample is equilibrated with a radioactive isotope of the element to be estimated, and the mixture of labelled and unlabelled atoms isolated, and the specific activity of the

isolated material estimated and compared with the specific activity of the added tracer. If the specific activity of the added tracer is S_1 c/m/mg, and that of the isolated material is S_2 c/m/mg, and the amount of tracer added to the sample initially is x mg, then the relationship

$$\frac{S_1}{S_2} = \frac{x + y}{x}$$ applies, where y is the amount of the sought for element in the sample in mg, whence the value of y can be found. The units in which the quantities are expressed are arbitrary, as long as the specific activities and weights can be compared as shown. Further more it is quite possible to invert the role of the activity in the chemical separation process, and to separate an element for, say, a colorimetric estimation, and to estimate the yield of the separation in terms of the return on added radio-tracer which requires the presence of a weight of the sought element insignificant in comparison to that present in the sample analysed. Thus activation analysis forms a major portion of the field of application of radio-isotopes and the associated techniques in analytical chemistry.

A.5. Applications of Activation Analysis:-

The fields to which activation analysis has been applied arethose where sensitive analysis is a prerequisite. Thus it has found application in medicine and the biological sciences generally, in criminalistics, in geology and geochemistry and in the technologies on which modern industry is founded.

Industrially activation analysis has applications in the analytical work associated with the semiconductor industry (130). The sensitivity of the method is made use of here, because very low impurity levels have

pronounced effects on the properties of the semiconductor material. Another field of application is the commercial geochemistry associated with petroleum deposit exploration and discovery (31), and the technique also finds application in product quality control in the same industry. (60).

In metallurgy activation analysis has been applied to the investigation of the composition of alloys (66) and is of great value in the steel industry is the determination of oxygen in steel (38). Activation analysis is also used in the preparation of standard materials (114). The routine use of activation analysis for inorganic material in industrial processes is not paralleled in its application to biological material.

Aside from the tissues of living organisms, the term 'biological material' is applied here to the habitat of the organisms as well as to the organisms themselves. In addition, applications to problems of forensic science are included. In biological analyses it is convenient to discuss two types of activation analyses. First there is the analysis designed to estimate one element at a time. Second there is one form or another of multi-element analyses.

In the course of an exhaustive study of the trace element metabolism of plants, Bowen and Cawse (20, 21) devised and tested activation analysis methods for a number of elements, the elements being selected by the line of enquiry pursued by the authors. The same authors used activation analysis to study the relationship between available tungsten and molybdenum and the concentration of both elements in different parts of the tomato plant (15, 22) and suggested that

tungsten was probably not essential to this plant, but might partially replace molybdenum in its function if the latter were not available.

An unusual application of activation analysis was the work of Weidinger and Jahn (165). In the course of an ecological study these workers labelled moth pupae with dysprosium, by including it in their diet. The mature moths were released and allowed to distribute themselve among the normal moth population. It was a simple matter to demonstrate the presence of those moths which had been exposed to dysprosium in a group of insects captured in any particular locality. The identification merely requires the activation of the moths one at a time, and the presence of induced dysprosium activity is easily detected in the labelled insects. This experiment demonstrates two principles. First it uses a stable tracer technique where the distinguishing label is non-radioactive and cannot harm the labelled object in any way, but is readily made observable by subsequent activation. Second, consideration of the number of labelled moths released, and the proportion of the moths collected subsequently which turn out to have been labelled, together with the number of moths collected for analysis permits estimation of the total number of moths occurring naturally. This is calculated in a similar fashion to that employed in an isotope dilution analysis.

Trace elements are of great interest in the field of dental medicine, because there are indications that small amounts of certain elements exert a profound effect on the nature of teeth in human and other species (4). Although the mechanism of dental decay is not understood, the work of Hadjimarkos and Bonhorst (63) (on selenium), and Ludwig et al (87) (on molybdenum and titanium), suggest that these elements may have some

effect on the incidence of dental caries due to their presence or absence in the diet. Better known is the reduction in caries incidence in children when the drinking water is treated with fluoride. The contribution to be made by activation analysis to this field lies in the sensitivity of the method, which will not only permit the estimation of trace elements in single teeth, but even permits the analysis of individual parts of a single tooth. One approach is that of Smith and Nixon (98,99) who do in fact analyse several sections from a single tooth. Sjoremark and Lundberg, on the other hand, (129) use a gamma spectrometric method to estimate seventeen elements simultaneously, but with much reduced sensitivity.

Activation analysis has found several applications in medical science. Koch et al (79) made use of the technique, not only to corroborate results obtained by other analytical techniques, but also to achieve analyses which were not possible by any other techniques, in their studies on the normal trace element content of human tissue. Again it is found that there are two types of analysis. First, there are analyses for a single element, such as the work of Tobias et al, and Sølvsten (155, 142), or the analysis of small biopsy samples, as the work by Westermark and Sjostrand (167), where samples as small as 3 mg in weight were successfully analysed. Banks et al (5) report successful activation analysis of blood for zinc using samples weighing about 80 mg, where the estimation by classical chemistry (the method of Valee - 158) required samples of about 3 g weight. Bowen (16) describes a simple method for estimating manganese in blood, using activation analysis with a simple chemical separation. The possibility of estimating the manganese activity without

chemistry is discussed, and the interference by iron which complicates the neutron activation process in blood are also discussed. Smith (133, 134) describes the normal arsenic content in a number of human tissues. Further analyses on the skin, hair and nails of patients suffering from a number of skin diseases for the same element did not, according to Girdwood Ferguson, Dewar, and Smith (53) indicate that arsenic was associated with these conditions, but the work does provide an example of the potential of the activation analysis technique. Comar et al (34) describe activation analysis methods for the estimation of copper, manganese, and zinc in biological materials and Pijck et al (108) give alternative methods for these elements, and also a method for cobalt for application to blood serum.

Spencer et al (146) advocate the use of activation analysis for the routine determination of blood electrolytes. These workers point out that the flux necessary may be achieved with a small neutron generator, or even an isotopic source, which, unlike the reactors used in trace element work, could be set up in a number of laboratories. The same workers describe a non-destructive method for the estimation of sodium in blood serum (147). The relationship between sodium and phosphorus content of cervical mucus, in relation to the menstrual cycle, was investigated by Odeblad et al, using a deuteron activation technique (102). The ease with which activation analysis can detect sodium (and potassium) was utilised by Keynes and Lewis (85) in their investigation of the change in the concentrations of these elements in nerves from squid and cuttlefish between the rest state and immediately after stimulation. It was possible to achieve this analysis non-destructively.

Proton activation, coupled with autoradiographic detection, permitted the estimation of O^{18} in chromatograms of alcohol extracts of algae, in an investigation of the mechanism of photosynthesis by Fogelstrom-Fineman et al (45). The same means of detecting the induced activity was used by Edwards (43) in his investigation of the distribution of B^{10} in mouse brain tumour. Benson (10) discusses the application of chromatography as a pre-irradiation treatment to minimise interference, and increase sensitivity, as well as improving the selectivity of the activation analysis technique. Neoplastic disease provides another field in which activation analysis is used. Tobias et al (156) compare the amounts of copper cobalt and zinc in the nuclear and cytoplasmic portions of neoplasms, and the effect of the neoplasm on the metabolism of these elements. A similar study was carried out on lung cancer by Kennedy et al (74), who reported that arsenic is concentrated in neoplasm without regard to the use of tobacco. The same authors describe the use of activation analysis for silicon for diagnostic purposes. Samsahlet al (121) report a method for the simultaneous determination of thirty elements in cancerous and non-cancerous human tissue. These last workers have developed a complex chemical separation based on anion exchange, and have applied the technique, not only to their study of cancerous tissue, but also to normal blood and tissues (26), and to a comparison of the trace element content of the urine and blood of uraemic patients in comparison with normal values (27).

Strontium and barium are two elements whose metabolism has acquired some importance, as fissile material produces long lived active isotopes

of these elements, it is necessary to know if they have a rapid turnover in the body or not. Early spectrographic techniques produced conflicting results for the strontium content of bone, and the general view was that barium was not present in the human skeleton at all. Sowden and others (145, 143, 144), using the activation analysis technique of Raymond and Harrison (65), demonstrated the presence of 7 and 100 p.p.m barium and strontium respectively in ashed bone. They were also able to measure the barium and strontium content of soft tissues, and in particular to show the concentration in the choroid tissues of cattle of barium and to a lesser extent strontium. The other members of the alkaline earth group of elements, calcium and magnesium, can also be determined by neutron activation analysis. The advantage of the activation technique is not significant for calcium, but for magnesium the activation method eliminates interference by calcium. Bowen et al (24), and Martinez-Duncker (91) have both published analytical methods for the neutron activation analysis of these elements.

Forensic science is a discipline which tends to make use of the latest techniques from a wide range of scientific sources, and activation analysis has been added to the techniques used in this type of work. (Smith and Guinn, 140 and 59). Sometimes single element analysis, with complete radiochemical separation is used, such as the work of Smith on arsenic. The sensitivity of the activation analysis technique is exploited to the full, not only in the analysis of single hairs, but even in the analysis of millimetre lengths of hair. Sectional analysis of this kind, or even rather easier instrumental analysis with a scanning device, or by autoradiography, permits the estimation of the distribution

of arsenic along the length of a hair. It is possible, from this type of investigation, not only to distinguish victims of attempted or successful poisoning from normal subjects, or from subjects who are exposed to arsenic in their work, but it is even possible to distinguish between chronic and acute exposure (135). The same technique was applied to hair taken from Napoleon immediately after his death, and the amount of arsenic found, in comparison with the normal range of arsenic from 1,000 random samples, indicated that arsenic had been administered to Napoleon (47). Another important development is the technique of gamma spectrometric analysis of hair following different periods of irradiation and decay, to evaluate non-destructively the concentrations of a number of trace elements in a small sample, often a single hair. Perkons and Jervis (104) discussed the proof of identity of an individual hair sample, taking account of such possible effects as change in trace element content of the hair, with location on the scalp, and variation of trace element content with time. One conviction has already been obtained based on evidence of this kind. Bate and Dyer (7) have also investigated hair individualisation.

The investigation of trace element content as a means of establishing identity can also be applied to such materials as paint, oil, soil and paper (50). The success of such analyses depends on the fact that the trace elements, on which the proof of identity rests, appear in the material analysed by accidental inclusion in the course of the manufacture of the material. Since no deliberate attempt is made to control the amounts of these elements present, their concentrations in paint, for example, will not only vary from manufacturer to manufacturer,

but also from batch to batch from a single source. Discussion of the techniques involved is to be found in the work of Guinn(59), and Bate et al (8). The same technique can be used to determine the source of narcotic drugs (104, 105), because the trace element content of the drugs depends on the trace element content of the soil in which the raw material was grown.

Certain industrial processes are accompanied by the evolution of toxic materials, and it is vital that the plant workers are adequately protected from the industrial hazards to which they are exposed. Smith (140) describes the estimation of the arsenic content of the hair of a process worker in a sheep-dip factory, which was of the order of 300 p.p.m., compared with a normal value of less than 1 p.p.m. Nixon and Smith (100) describe the application of activation analysis to hair and nail samples from a dentist and his assistant, who complained of having th characteristic symptoms of chronic mercury poisoning. The analytical results showed that they were in fact suffering from exposure to mercury vapour, and investigation of their work place revealed a large amount of spilled mercury close to a heater. Activation analysis has been applied to the estimation of pesticide residues in foodstuffs, such as the work of Guinn and Potter (62), on the estimation of bromine fumigants for soil purification. Guinn (61) has described the application of activation analysis to the determination of mercurials in seed wheat, to demonstrate the attempted illegal addition of the treated wheat to the pure material.

It is clear that activation analysis as applied to medical science, including dental medicine, toxicology and criminology, as well as biochemistry, has a valuable contribution to make to the progress of these

disciplines. Applications may involve activation either by neutrons or by charged particles. Often, the activation analysis technique is chosen for the estimation of a single element because of its sensitivity. Again the technique may be adopted because it offers greater specificity and freedom from interference by other elements than any other analytical technique. A further advantage is the possibility of multi-element analysis, either using radiochemical techniques to separate the induced activities, or adopting the non-destructive approach, which permits analysis without the destruction of the sample. Each approach has advantages and disadvantages, and the method chosen depends on the problem in hand and the equipment available.

(B) NEW ACTIVATION ANALYSIS METHODS.

B.1. Introduction:-

The approach Adopted in the Development of a New Activation Analysis Method.

Before attempting the analysis of a material for a particular element or group of elements, by activation analysis, it is essential to determine that activation analysis will have some real advantage over the classical methods available. Consequently time is well spent in examining the literature for other methods applicable to a particular problem, noting the sensitivities available, and the interferences to which each technique is liable.

Having decided that activation analysis may have something to offer, it is necessary to consider the nuclear reaction to be used in the activation process. Activation by charged particles will not usually be useful, partly because these are expensive to use, and partly because the energy transferred in the interaction of these particles with a target is very large and makes sample presentation very difficult, at least for biological materials. Further, these charged particles have very low penetrating power, so that if a homogeneous flux is to be maintained throughout the sample, then the latter must be very thin. Activation by photons, (γ, n) reactions, is used for activation analysis but the sensitivities available are not well documented. In any case the cross-sections for this type of reaction are small, and the photons themselves are not very readily available. Finally there are the neutron induced reactions. As pointed out previously, the most

profitable activation technique is that of neutron capture using thermal neutron sources, such as reactors, neutron generators, or isotopic sources.

In addition to considering the means of activating the desired element, it is necessary to consider what other activities will be generated in the sample during activation. Neutron capture is fairly selective in terms of the elements which are activated, but even in material from animal sources the majority of the induced activity is that of Na^{24}, Cl^{38} and P^{32}. However, in the neutron flux from a reactor, although a large proportion of the available neutrons are of thermal energy, a significant flux of higher energy neutrons is present. Borg et al (14) greatly reduce the Na^{24} and Cl^{38} activity induced in blood serum without reducing the activation of manganese, permitting the instrumental detection of the latter. This depends on the fact that manganese has a large resonance capture cross-section for epi-thermal neutrons. Attenuation of the thermal neutron component of the flux by wrapping the samples in cadmium foil permits the resonance activation of manganese, while suppressing the activation of sodium and chlorine.

More significant than the production of activities other than that desired is the possibility of ~~the~~ interfering nuclear reactions producing the desired activity from the wrong target element. This gives erroneous results, which can only be corrected by evaluating the amount of interfering target element present in the sample, and also its contribution to the desired activity. Fritze et al (48) describe an activation analysis technique for the estimation of copper, which allows for interference by the reaction $Zn^{65}(n,p)Cu^{65}$, and eliminates it.

The presentation of the samples for irradiation should be considered. In general, pre-treatment should be kept to a minimum. It is, however, convenient to dry material for analysis, bearing in mind the possibility of contamination by the drying agent. This permits the use of aluminium foil for packing soft tissues, bone and even blood serum. It is better to use polythene containers if this is possible, either in the form of sachets or small specimen tubes, subject to the effect of the irradiation on the polythene. These containers have the advantage that they can be heat-sealed. Liquids, such as urine samples, or comparator standard samples can be presented either in sealed polythene tubes for short irradiations, or sealed silica tubes. These different packing materials are used to contain individual samples. For the actual irradiation in a reactor the material is always packed in another container, made of aluminium or polythene depending on the duration and temperature of the irradiation.

The next step after the activation process is the separation of the induced activities. The activation process is again involved here, as the amount of interfering activity, if of longer half life than the desired activity, is reduced by activating for the minimum time compatible with the attainment of the desired sensitivity. Short lived interferences may be eliminated by allowing them to decay before the desired activity is assayed. The detector used is governed by the nature of the activity. A geiger counter offers little resolving power, but better sensitivity due to its lower background compared with a scintillation detector. The latter is not applicable to the detection of certain activities which decay without gamma ray emission. Smith

(140) describes the resolution of a simple mixture of As^{76} and Na^{24} counted with a Geiger counter, both by decay curve analysis, and by differential absorption of the emitted beta particles by aluminium filters. These techniques are of limited application, although they have been applied to the resolution of mixtures of Na^{24}, K^{42} and P^{32} (11, 112).

In general chemical separation will be necessary to eliminate interfering activity and simplify the assay of the sought for activity. Smith (140) has reviewed the processes involved in a chemical separation for activation analysis, under the following headings:-

(a) Digestion of Sample:- The process of bringing the active sample into solution and equilibrating the constituent activities with inactive carrier material is usually accomplished by a wet digestion technique. Of the various mixtures used the most popular are mixtures of sulphuric and nitric acids, or nitric acid alone. Fusion techniques such as that of Kaiser and Meinke (70), using sodium peroxide, are occasionally found.

(b) Separation Techniques:- The standard methods mentioned in Section A.3. can be tabulated as in Table B.1. and compared on the basis of specificity, the time they require, and their applicability. The most selective separation process in general use is that of distillation. This technique is rather limited in its application due to the relatively small number of volatile inorganic compounds, which are confined largely to the halogens and certain halides, hydrides, and oxides. The mass spectrometer, though obviously extremely selective, and in principle widely applicable, is not in fact a serious competitor because of the problems of presenting the sample in a form suitable for the operation

of the spectrometer. The most widely used technique is that of precipitation, with solvent extraction a close second. The latter is less favoured for routine work involving large numbers of samples due to the risk of carrying contamination from sample to sample if apparatus is used more than once in a single analysis. Cross-contamination can be avoided by the use of clean glassware for each sample, but the amount of equipment required for an analysis then tends to become unmanageably large. Chromatography, though time consuming, in the form of ion-exchange chromatography is the basis for several multiple element analysis schemes.

(c) Recovery Calculations:- The yield of the radiochemical process may, as stated in section A.3. above, be determined by any of the methods of classical analysis, i.e. colorimetry, gravimetry, or even by a second activation analysis. One further means of accomplishing the same result is to add a radioactive sample of the element to be separated. This added activity must not interfere with the estimation of the activity induced in the sample by the activation process, and must not be produced in the sample. In addition, it is usually relatively long lived in order that the sample activity may decay completely before the added tracer is measured to give the yield of the separation. This has been used by Smith (136) in determining manganese.

(d) Presentation of Separated Material for Counting:- Depending on the nature of the final step of the separation procedure, the activity to be measured may be either in liquid or solid form. Precipitates may be transferred as a slurry, or suspension, in water, or better in some more volatile liquid, to a metal counting tray whose size and shape is governed by the counting assembly used. An alternative is to filter the solid

material off, using a filter stick. The solid material is washed in the usual way, and the paper and precipitate are mounted together on a counting tray or planchet. Sometimes it is of advantage to fix the precipitate to the counting try, to minimise losses and reduce the hazards of dropping prepared sources, by treating the mounted material with polyvinyl acetate solution, which is then evaporated to dryness. The addition of the plastic material must follow any weighing of the separated material. Evaporation of solvents in source preparation is most conveniently managed by the use of an infra-red lamp which can produce temperatures up to about $90^{o}C$. In preparing sources from solid materials, it is essential, especially for beta counting, to ensure that the precipitate is distributed evenly over the mounting surface to avoid anomalies in the counting efficiency of the detector due to self-absorption of the emitted radiation. In general precipitates for counting should not contain large amounts of heavy elements which increase their density and hence the self-absorption of the source.

Both beta (Geiger) counting and gamma scintillation counting can be carried out on liquid samples. The liquid Geiger counters have the disadvantages that the sample is placed in contact with the counter assembly, so that contamination is a hazard, and the construction of the detector restricts the lower energy limit of beta particles which can be detected in comparison with an end window Geiger counter. For scintillation counting with a sodium iodide crystal, the sample is usually simply placed in a test tube which fits in a well in the crystal, or in some other container which fits over a small crystal. In all cases where liquid samples are to be counted it is necessary to control

the volume of solution used with some care, because the efficiency of the counter is strongly dependent on this factor.

(e) Testing the Method:- As in any analytical technique it is necessary to establish that the method adopted is operating as expected. In activation analysis the examination of a new method falls into two parts. First it is ascertained that the desired radiochemical purity is attained. This is achieved by the activation and processing of a representative batch of samples and a standard comparator. The correct function of the separation on a qualitative basis is demonstrated by the identity of the gamma spectra of samples and standard, and their decay constants. Decay curve comparison is much more sensitive to the presence of extraneous activities. The determination of the maximum beta energy may also be applied to test the purity of the activity isolated from the samples.

Quantitative evaluation of the method is approached in the first instance by the analysis of standard samples prepared in the laboratory. The simplest test is the attempted analysis of simple solutions. The next step may be to attempt the same analysis, but in the presence of a suspected interference. Lastly the best test if the material is available is the analysis of a standard material of known content. The problem here is that homogeneous biological material is difficult to obtain. One source of such material is the dried kale prepared by Bowen ([23]), under rigorous conditions to control contamination and ensure homogeneity. This was demonstrated by a series of tests. Apart from this material the best alternative is a biological fluid of some kind. This is not a very satisfactory test for a method which is to be applied to solid materials. In any case the trace element content of the

standard material has to be determined in some way to provide a basis for comparison.

The points to be considered in setting up an activation analysis method fall under a series of headings. First the activation process must be decided. Second the manner in which the mixture of induced activities is to be resolved must be selected, the choice being between a chemical process, or a purely instrumental approach, or a judicious combination of the two. Finally the entire procedure must be tested qualitatively and quantitatively.

B.2. Determination of Antimony in Biological Material.

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B.2.1. Requirement to be Met by the Method.

The method is required for application to the estimation of antimony in a variety of biological materials, not only to demonstrate the amount of antimony normally present, but also to investigate the mode of action of antimonial compounds used in the treatment of bilharziasis. The method is required to deal with large numbers of samples, and it is therefore desirable that it be manipulatively simple, which also permits its use by relatively unskilled operators.

B.2.2. Available Methods for Estimating Antimony.

Antimony can be detected by colorimetric methods (Sandell, 104) with sensitivities in the microgram range, of which the most sensitive technique is the estimation of Sb^{V} using rhodamine - B. Other colorimetric methods are the tetraiodoantimonate estimation devised by Washington (164), or the molybdate colour method of Matulis and Guyon (92).

The limit of detection quoted for the latter is 0.02 p.p.m. but like the rhodamine-B method the colour is very unstable. Both of these methods estimate Sb^{III}.

Polarographic methods are also available for the estimation of microgram amounts of antimony (64, 115). Both the colorimetric and polarographic methods require the elimination of interfering elements, and the quantitative manipulation of small amounts of antimony. A simpler technique is the spectrographic technique of Kinser et al (76), which permits the estimation of 50 p.p.m. Sb in 2 mg of ashed biological material applied directly to the graphite electrode of a d.c. arc spectrograph. This is equivalent to the estimation of 0.1 micrograms of antimony.

Activation of naturally occurring antimony for 3 days at a thermal neutron flux of $1.2 \times 10^{12} n/cm^2/Sec$ produces a specific activity of 290 mc Sb^{122}/g. Assuming a detector efficiency of 10% of the total number of disintegrations, the detection of 0.5 c/s corresponds to a sensitivity of 9.3×10^{-10} g Sb. Thus potentially neutron activation analysis can detect 1/20th of the amount of antimony detectable by the colorimetric method of Matulis and Guyon. A convenient amount of material for a single analysis is about 20 mg, and assuming that the level to be determined is of the order of 0.1 p.p.m. then the method is required to determine 5.0×10^{-9} g Sb. If this limitation of sample size is accepted then activation analysis is the only technique sufficiently sensitive to achieve the analysis.

B.2.3. Thermal Neutron Activation Properties of Antimony.

The capture of thermal neutrons by naturally occurring antimony results in the formation of two active species, Sb^{122} and Sb^{124}, as shown in Table B.2. Most of the activity is due to the shorter lived Sb^{122}. Also given in this table are reactions other than the desired neutron capture reactions which can produce radio-antimony. Neutron capture followed by beta decay produces Sb^{125} from Sn^{124}. This interference is entirely negligible due to the low cross-section of the reaction and the low tin content of biological material.

Of greater significance are the interfering reactions brought about by the activation of elements adjacent to antimony in the periodic table by fast neutrons accompanying the reactor thermal flux. The formation of Sb^{122} from the (n,p) activation of Te^{122} can be neglected as a possible source of error in the analysis of biological material due to the low cross-section of Te^{122} for this reaction, the very low concentration of tellurium in the material analysed, and the ratio of fast to thermal neutrons in the flux, of the order of 1:10 (30). On the other hand the reaction $I^{127}(n,\alpha)$ Sb^{124} in certain circumstances constitutes a source of error. Again the low ratio of fast to thermal neutrons in the flux, and the cross-section for the reaction render it relatively unproductive, but certain biological materials, especially thyroid tissue, are rich in iodine, while not especially rich in antimony, so that an appreciable portion of the total antimony activity could be due to the (n,α) reaction. An upper limit for this effect may be calculated as follows:-

In Table B.2. the specific activity of Sb^{124} produced per gram of iodine after irradiation for three days at a thermal neutron flux of 1.2

x 10^{12} n/cm^2/sec, assuming that the accompanying fast neutron flux has 1/10th this value, is given as 8.0μc/g. The cross-section value used for this activity calculation applies to activation by 14.3 MeV neutrons, and since the cross-section decreases with neutron energy, and only a fraction of the reactor fast neutrons have this energy the calculated activity is very much an upper limit. Thyroid tissue contains 350 p.p.m. iodine (44), and assuming an antimony content of 0.1 p.p.m. the ratio of activity from neutron capture activation of antimony to that formed by the (n,α) reaction on iodine is given by $\frac{295 \times 0.1}{8.0 \times 350 \times 10^{-3}} \approx 10.$ It is shown by experiment that activation of ammonium iodide in the same conditions as those used for the analysis results in the formation of an antimony specific activity per gram of iodine which is 2.5 x 10^{-6}% of that produced in an antimony standard. The activated iodide is processed and counted exactly as any other sample put through the antimony analysis, and therefore direct comparison of the activity generated in the iodide with that induced in an antimony standard is possible. The empirical activities obtained result in the ratio (neutron capture activity/interfering activity) of 1.14 x 10^4 for thyroid tissue. The difference between the calculated ratio and the empirical value is accounted for by the approximations used in the calculation of the former. Overall the proposed activation technique is free from interference even in the case of samples rich in iodine, such as thyroid samples. The precise extent of the interference from iodine depends on the relative amounts of thermal and fast neutrons in the flux used, and on the energy of the latter. The energy profile of the available flux, and the degree of interference, depends not only on the type of reactor

used, but also on the position of irradiation of the samples within a given reactor. The effect of the decay of the samples between the end of irradiation and the time of measurement of the desired activity is neglected in this discussion, because it is negligible in practice.

B.2.4. Radiochemical Separation for Antimony.

After irradiation for three days at a thermal neutron flux of 1.2 x 10^{12} n/cm^2/sec, even after a cooling period of 30 hours, almost all the activity in activated biological material is due to Na^{24} and P^{32}. The desired antimony activity is quite undetectable against these major activities. Consequently some sort of chemical separation is essential, and the more complete the separation is, the greater the sensitivity of the complete activation analysis.

Typically such a separation is achieved in four stages. First the sample is mineralised and brought into equilibrium with a solution containing inactive carrier for the element to be separated out. Then follows an initial separation step designed to isolate the desired element from the bulk of interfering activity, the latter being discarded. The next stage is usually to eliminate specific interferences which are carried through the initial separation. Some sort of general decontamination is carried out making use of a scavenging agent usually in the form of a bulky precipitate. The last step is the separation of the desired element in a suitable form for the activity estimation. This scheme is the minimum possible for any kind of complete separation, but very often if the sought for activity can be resolved from a fairly complex mixture of activities, using gamma spectrometry, then the

separation can be reduced to the first two steps only. From Table B.2. it is seen that Sb^{122} has abundant beta emissions over 1.0 MeV which would make the isotope readily detectable with a Geiger counter. It also possesses a 66% abundant gamma ray at 0.57 MeV suitable for gamma spectrometry. It is better to use the beta emissions for detection purposes due to their greater total abundance, despite the loss of resolution and the more stringent radiochemical separation this entails. Gamma spectrometry is invariably less sensitive than Geiger counting for an isotope with about equally abundant beta and gamma emissions due to the very much higher background of the spectrometer. The properties of the emissions of Sb^{124} need not be considered as this isotope contributes relatively little to the total antimony activity under the irradiation conditions used.

The chemical and physical properties of antimony useful in radiochemistry, are dealt with in a monograph by Maeck (89). The chemistry of antimony and its compounds is dealt with exhaustively by Sidgwick (127).

In the digestion processes used in the destruction of organic material the essential features are the application of heat to drive off volatile material, and the use of an oxidising agent to eliminate the carbonaceous decomposition products. Antimony presents a problem in the selection of a suitable digestion technique, due to the volatility of several of its compounds, especially those of Sb^{V}. The choice lies between a fusion technique using sodium peroxide or silver nitrate or one of a number of wet ashing techniques. These include sulphuric and nitric acids in various proportions, replacing nitric acid by permanganate or

dichromate, or mixtures of nitric and perchloric acids. Although the fusion techniques can be accomplished rapidly it is not easy to carry out a large number of digestions simultaneously using this technique. Of the wet ashing techniques the perchloric acid method can be ruled out immediately because of the risk of losing volatile antimony chlorides. The mixture employed by Smith (133) of 5:3 (v/v) 16M nitric acid and 18M sulphuric acid, using the digestion flasks employed by the same author, which have relatively long glass necks to act as reflux condensers, is satisfactory in principle for antimony. It is found, however, that the complete elimination of nitric acid necessary for the success of the next step in the separation is a lengthy process, and the added antimony carrier tends to precipitate as a white solid which is difficult to re-dissolve completely after the end of the digestion.

A more rapid technique is the use of sulphuric acid. Using 36N acid in conical beakers it is found that up to 1 g of dried tissue can be destroyed completely in 10 minutes provided the acid is heated till fumes appear. The product of this digestion is a charred mass which must be cleared before the separation process continues. This is done by the addition of a small amount of an oxidising agent. Nitric acid, sodium nitrate and hydrogen peroxide are all suitable. The next step is best carried out in the absence of oxidising agents, and hence it is desirable that all the excess oxidant be removed before the separation proceeds. Hydrogen peroxide is readily decomposed by heat. If either of the other reagents is used then the addition of a little solid sodium sulphite serves to reduce the residual oxidant, and helps to retain the antimony in the digest in the less volatile tri-valent oxidation state.

The amount of sulphuric acid used is governed only by the consideration that its volume is great enough to dissolve samples of normal size as quickly as possible. In practice it is found that 2 ml is adequate for samples up to 100 mg in weight. The amount of oxidising agent is not critical. It is convenient to add the oxidant in small portions until the digest is clear and pale yellow in colour. The inactive carrier antimony is added to the sample in the digestion beaker before the sulphuric acid. The desired carrier concentration of 20 mg/ml is attained by dissolving potassium antimony tartrate in approximately 6N hydrochloric acid. The addition of the carrier to the sample before the commencement of the digestion process is intended to facilitate the equilibration of the radioantimony from the sample with the added carrier, and to minimise the loss of the former.

The next step requires the transference of the digest to another container. Since all antimony salts are hydrolysed, at least partly, on dilution, with the formation of insoluble basic salts, simple dilution of the digest is not possible. It is found however, that the precipitate formed by the dilution of the digest with 3 to 4 ml of water is re-dissolved by the addition of 1 ml of 11M hydrochloric acid, due to the formation of a chloro complex of antimony. Thereafter the digest may be transferred to another container, and the digestion beaker rinsed out into the same container. The solution can be diluted as necessary without hydrolysis products precipitating.

The next step in the process should eliminate as far as possible all activities other than antimony. The possible procedures are limited to volatilisation of either the chloride or hydride, solvent extraction of

the antimony complex formed with any of the halogens, or precipitation. Solvent extraction can be eliminated as a possibility merely on the grounds that this type of step is not suited to a routine determination where a single run is intended to cope with large numbers of samples. The technique is more suitable in handling a short lived activity, where the speed and specificity of the technique are at a premium.

A volatilisation or distillation separation step is an attractive possibility initially, because such a process can be very selective, despite the time it requires for its completion. In the case of antimony, however, although wide use has been made of its volatile compounds in radiochemical separations, the degree of specificity is rather poor, and the technical difficulties rather great. Distillation of antimony as the pentachloride or pentabromide requires the presence of large amounts of the halogen, and relatively high temperatures. The evolution of stibine, although it has been used by Abdel Rassoul et al (1) as part of an activation analysis estimation of antimony, requires drastic conditions to permit the reaction to occur without the deposition of metallic antimony. Arsenic is evolved along with the stibine but can be separated from it by the different stabilities of the two hydrides. Greendale and Love (55) describe the separation of radioantimony from fission products in the same manner using zinc and sulphuric acid. The production of stibine can also be achieved electrolytically. Contamination of the separated material with arsenic, the complexity of the technique, the time required, and the risk of low yields due to the deposition of metallic antimony, all make stibine evolution a poor choice for the purposes of a routine analysis.

Antimony forms many compounds insoluble in water, which might be suitable for a precipitation separation. Aside from precipitates formed with organic reagents which are in general more suitable for a final precipitation step because of their bulk, there are several possibilities. Insoluble oxy-compounds may be precipitated by the hydrolysis of antimony salts, but the composition of these compounds is very variable and the completeness of the precipitation is difficult to assess. Both tri- and penta-valent antimony can be precipitated as the sulphide, and the standard procedures of qualitative analysis can be adapted to purify the precipitated material satisfactorily for activation analysis purposes. Such purification, for example as described by Bowen (25), requires several steps, making the separation rather tedious for routine application. The remaining alternative is the precipitation of antimony as the metal by a suitable reducing agent. The advantage of such a process is that after the oxidation process used in the mineralisation of the sample it makes the carrier pass through all the available valency states. This ensures equilibration between the carrier and the radioactive antimony produced by the activation process. The best reagent for this purpose is chromous chloride, mentioned by Maeck (89) and used by Kennedy et al (74) for the precipitation of elemental arsenic. The reagent itself is a strong reducing agent, sensitive to oxidation by air. Consequently the material available commercially in the form of a 1M solution is kept under an air seal of alcohol. In practice it is found to be impossible to use the reagent without contaminating the solutions with the alcohol, which makes the precipitated material very difficult to manipulate. The reagent can be prepared in the laboratory

by the simple expedient of treating an acid solution of a chromic salt or chromic oxide with metallic zinc. Small amounts of the reagent can be prepared just before use in this way. The best method of preparation is by the reduction of chromic chloride in 1.5 M sulphuric acid using a Jones' reductor (159). The product is stored under an atmosphere of hydrogen to avoid the use of the amyl alcohol. By this means up to 2.5 litres of reagent at a strength of about 1 M can be prepared, and available for use as required, free from contamination by organic preservative.

The best conditions for the precipitation of antimony by chromous chloride are determined by tracer studies using Sb^{124}. It is simple to evaluate the amount of antimony left in solution after a precipitation carried out under a particular set of conditions. Tracer is placed in the test tube along with inactive carrier, the precipitation carried out and the activity remaining in the supernatant determined using a Geiger counter accepting liquid samples. These experiments (Table B.3. and Fig. B.1.) show that the only requirement for the complete precipitation of antimony is the presence of excess reducing agent in an acid solution of antimony. The temperature of the antimony solution before the addition of the reagent and the time and temperature of digestion of the precipitate have no effect on the efficiency of the chemical process. These factors influence the physical nature of the precipitated material, especially the size of the particles of which the precipitate is composed. The precipitate must be sufficiently aggregated for its centrifugation to be complete even after washing with water. This is accomplished by adding the reducing agent to a hot solution and

continuing heating for 5 minutes. If heating is continued further the only result is the evolution of gases from the solution by the action of the excess reducing agent. This makes it impossible to spin down the precipitate until the solution and precipitate have been allowed to cool for some time.

Antimony is not the only element which is precipitated under the conditions used. As, Bi, Cu, Pt, Au and Hg are also precipitated as shown by simple qualitative tests. Similar tests show that Ba, Ca, Cd, Co, Cr, Fe, I, K, Li, Mg, Mn, Ni, P (as ortho-phosphate), Pb, Sr, Sn, V and Zn are not precipitated. A quantitative evaluation of the degree of interference by copper, arsenic and mercury has been made using the appropriate tracers. Copper and mercury are precipitated completely whether carrier is present or not. In the case of arsenic, however, when the total amount of arsenic present in the antimonial solution is less than a few micrograms only 1 - 5% of the arsenic is precipitated along with the antimony. This is determined by the arsenic activity remaining in solution after the precipitation process is complete. It is usually possible to improve the selectivity of a precipitation step by diluting the interfering activity with stable carrier, assuming that the amount of arsenic precipitated depends on the precipitate formed and not on the amount of arsenic present. Then the added carrier not only reduces the specific activity of the arsenic in the solution, but also the contaminating activity in the precipitate. This cannot be done here, because chromous chloride produces a precipitate from an acid solution of arsenic carrier. Consequently the effect of the addition of arsenic carrier to the solution, before the precipitation of antimony, is to increase the

contamination of the antimony precipitate by radioactive arsenic. The behaviour of arsenic in trace amounts is anomolous. Further purification is necessary before the isolated antimony activity is estimated. For this purpose some means of redissolving metallic antimony is required. There are only three possibilities. One is the use of hot sulphuric acid as advocated by Maeck (89) and Greendale and Love (55), but this is found to be unsuitable. The latter authors have used tartaric acid for the same purpose but again this is unsuitable for this analysis. It is suggested that the failure of these reagents is due to the physical properties of the precipitated antimony. The remaining possibility is the use of a solution of a halogen. In practice the most convenient variant of this technique is to suspend the antimony in 11M hydrochloric acid, and produce free chlorine by the addition of a few drops of 30% (w/v) hydrogen peroxide. The precipitate is allowed to dissolve in the cold to avoid losing volatile chloro-compounds of antimony. The solution formed is contaminated with the activities of copper, mercury, arsenic, bismuth and gold. Qualitative tests show that platinum only partially dissolves in the hydrogen peroxide/hydrochloric acid mixture. Experiments with Sb^{124} show that up to 10% of the precipitated antimony may be lost in the dissolution process, despite the precautions taken.

The problem which remains is the elimination of arsenic, copper, mercury and the other contaminants. Arsenic presents the greatest difficulty here because of its chemical similarity to antimony. Smales and Pate (131) describe the precipitation of arsenic by reduction to the metal using ammonium hypophosphite. It is found by both qualitative and

quantitative (tracer) experiments that this reagent did not precipitate antimony. The optimum conditions for the elimination of arsenic are established by tracer experiments (Table B.4 and Fig. B.2.) The requirements are, the presence of an excess of reducing agent in a strongly acid medium and the application of heat to complete the precipitation. The same reagent accomplishes the elimination of mercury with or without added carrier, and also of gold and platinum. Gold and mercury are reduced to the metallic state, but platinum is precipitated in the form of a yellow compound, presumably platinous chloride. Any silver which may have been precipitated by chromous chloride is present at this stage as the insoluble chloride, and is discarded along with the arsenic precipitate, although this element will also be reduced to the metal by hypophosphite. The precipitation of arsenic by hypophosphite is a reaction with several peculiarities. First, anomalous results are obtained when tracer prepared by the thermal neutron activation of arsenic trichloride was used with arsenic trichloride carrier. It is found that the added activity is not precipitated completely although the added carrier is. This difficulty is overcome by adding the arsenic carrier to the precipitated antimony before the latter is dissolved by the addition of hydrogen peroxide. This arrangement permits the active arsenic to exchange and equilibrate with the added inactive carrier. Secondly, the precipitation exhibits an induction period of about 3 minutes. This is followed by the formation of a brown precipitate which on digestion with continued heating is converted into a crystalline black precipitate. To maintain the high acid concentration necessary for the completion of the precipitation, Smales (131) uses solid ammonium

hypophosphite. In the present method a saturated solution of either ammonium or sodium hypophosphite is suitable. The latter produces a white crystalline precipitate of sodium chloride when the reducing agent is added to the strongly acid arsenical solution, but the salt dissolves on heating.

The remaining contaminants are bismuth and copper. Bismuth, like arsenic, is chemically very similar to antimony and hence difficult to separate from it. In fact, no satisfactory method has been found. The most promising method is the precipitation of bismuth as the carbonate. This requires an alkaline medium. Antimony can only be retained in solution at pH's greater than 7 by complexing it with tartaric acid. Bismuth also forms a complex with this reagent and the insoluble carbonate is not then formed. In any case tracer studies revealed that large amounts of antimony activity precipitated even in the presence of tartaric acid. On the basis of the low thermal neutron capture cross-section of bismuth, 0.019 barns (2), and its low natural occurrence relative to antimony, the failure to exclude bismuth does not invalidate the method. The neutron capture product of Bismuth, Bi^{210}, is an α and β emitter, but does not emit gamma rays, so that if a sodium iodide crystal is used to detect the antimony activity by means of its 0.57 MeV gamma emissions any possible interference by bismuth is eliminated.

To eliminate copper, a standard precipitation used in many separations is the precipitation of cuprous thiocyanate. Copper carrier added to the supernatant from the arsenic precipitation is immediately reduced to the cuprous state by the excess hypophosphite. The addition of thiocyanate results not only in the precipitation of copper, but also of antimony sulphide due to the decomposition of the thiocyanate by the

The final step of the separation is required to leave the antimony in a suitable form for counting purposes. Much the simplest approach in a sequence of the type outlined above is to form a precipitate of some kind. The only technique which is compatible with the previous steps is a second chromous chloride precipitation. For the success of this step it is necessary to eliminate the excess ferricyanide from the precipitation of copper. This is readily achieved by the addition of excess cobaltous chloride, and the formation of insoluble cobaltous ferricyanide. The combined precipitates of arsenic, cuprous ferricyanide, and cobaltous ferricyanide are spun down and filtered off from the solution containing antimony and excess cobaltous ions. Antimony is then precipitated from the filtrate by the addition of chromous chloride.

For the determination of antimony in biological materials by activation analysis, a suitable separation scheme is as follows. The samples are wet ashed in the presence of antimony carrier, using sulphuric acid. Precipitation of antimony as the element eliminates the bulk of the unwanted activity. A succession of precipitations are then used to eliminate copper, mercury and arsenic. Finally antimony is presented in a form suitable for mounting on planchets and counting by a second chromous chloride precipitation. One element other than antimony follows this scheme, namely bismuth, but little bismuth activity is likely to be present in samples of biological material and moreover antimony and bismuth can be separated by a suitable choice of detector.

B.2.5. Practical Details and Experimental Evaluation of the Proposed Method.

(a) Preparation of Samples.

To minimise the risk of contamination, sample preparation is reduced to the minimum necessary for the convenient irradiation, and post-irradiation treatment, of the samples. Samples of hair and nail are packed in aluminium foil as received, except where contamination has already obviously occurred, when some attempt may be made to remove foreign matter by mechanical means. All other tissues, such as liver, lung, brain, etc. including blood, are thoroughly dried by storing them under reduced pressure in the presence of silica gel, for a period of three days to one week, and sometimes longer. The drying process is accelerated by decreasing the pressure to 0.1 mm Hg, and employing a cold trap filled with a mixture of powdered solid carbon dioxide and methanol. In this way drying time can be reduced to eight hours or less. For the best results it is advisable to freeze the samples before reducing the pressure, so that they are freeze-dried. The dried samples are then packed in aluminium foil in the same way as hair and nail. Normally samples weigh between 20 and 50 mg. Dilute aqueous solutions and samples of urine are irradiated in 100 mg aliquots weighed into silica ampoules 5 cm long with an internal diameter of 3 mm. These are sealed using a coal-gas/oxygen flame. The ampoules when packed are about half full of liquid, so that adequate gas space remains to prevent their destruction by the pressure of gaseous decomposition products formed during irradiation. The ampoules are easier to open on their return to the laboratory after irradiation if they are pulled out into a constriction about 1 cm from one end, thus providing a point at which they can be fractured without risk of spilling the contents. The comparator standard is packed in a similar ampoule. Such a standard is

usually either a dilute solution of the element to be determined, of accurately known concentration, or a small amount of a solid compound of the element. In view of the ease with which antimony compounds undergo hydrolysis in aqueous solutions, a standard solution of antimony requires frequent calibration, and hence this method of presentation is avoided. Instead, about 1 mg amounts of potassium antimony tartrate are used as standards. This compound is stable, and easy to handle in small amounts The fact that after irradiation considerable potassium activity will be present does not matter, because after irradiation the standard is dissolved in hydrochloric acid, diluted, and an aliquot of the diluted solution is processed in the same way as the samples, so that the antimon activity is completely freed from potassium.

Considering the half life of the principle activity induced in antimony, the maximum useful irradiation time is three days. The method of packing samples described above is suitable for irradiations carried out in the U.K.A.E.A. reactor B.E.P.O. at Harwell at a thermal neutron flux of 1.2×10^{12} n/cm^2/Sec, i.e. pile factor 12, provided samples and standard are packed together in a standard size aluminium irradiation container. The three inch long can (size 'A') normally used can hold up to 100 foil wrapped samples and a comparator. Alternatively up to 18 silica ampoules can be packed in a single aluminium can. The materials in which the samples and standard are packed may be different for shorter irradiation times, or for other reactors. For example, samples rich in antimony have been analysed after irradiation for 2 to 6 hours at the Scottish Research Reactor, East Kilbride, using polythene containers.

After an irradiation carried out in B.E.P.O. 30 hours delay occurs

between the end of the irradiation and the commencement of the chemical separation. This cooling time results in a four-fold decrease in the amount of Na^{24} activity induced in the samples, and permits the complete decay of the Cl^{38} activity. The major activities in the samples at the commencement of the chemical separation are, residual Na^{24}, K^{42} and P^{32}.

(b) Chemical Separation.

1. Reagents.

Antimony carrier - 20 mg Sb per ml, prepared by dissolving 51.8 g potassium antimonyl tartrate in 1 litre 6 N hydrochloric acid.

36 N sulphuric acid.

Sodium nitrate and sodium sulphite, or 100 vol hydrogen peroxide or 16 M nitric acid.

11 N hydrochloric acid.

Chromous chloride, approximately 1 N - prepared by reduction of a solution of 250 g hydrated chromic chloride in 1 litre 4 N sulphuric or hydrochloric acid (see above).

100 vol hydrogen peroxide.

Arsenic carrier - 10 mg As per ml, prepared by dissolving 13.3 g arsenious oxide in 1 litre 1 N hydrochloric acid.

Sodium or ammonium hypophosphite - solution saturated at room temperature.

Copper sulphate - 10% (w/v) solution.

Potassium ferricyanide - 10% (w/v) solution.

Cobaltous chloride - 10% (w/v) solution.

Acetone.

2. Transfer of Samples for processing.

Solid samples, packed in aluminium foil are transferred completely to 125 ml conical beakers and 2 ml 36 N sulphuric acid and 1 ml antimony carrier are added. Liquid samples are transferred by pipetting them from

the silica irradiation containers using disposable pipettes, and rinsing pipette and container with a solution containing the carrier and sulphuric acid in the same amounts as for the solid samples. Any un-used wash liquid is added to the digestion beaker after transfer is complete.

The tube containing the comparison standard is opened and the contents leached out with hot 11 N hydrochloric acid. Some antimony carrier may be added to the acid to ensure the complete extraction of the active antimony. After cooling, the solution is transferred with thorough washing to a 1 litre standard flask, which is then filled to the mark with water. A further dilution by a factor of ten is accomplished by the transfer of 10 ml of this solution to a 100 ml flask, which is also made up to the mark. An aliquot of the latter solution (1 ml) is transferred to a conical beaker containing antimony carrier and sulphuric acid as for the solid samples. Thereafter the standard is processed in exactly the same way as the samples. Although the exact concentration of the antimony carrier does not matter it is imperative, for the recovery correction applied in the comparison of the activities isolated from samples and standard, that the carrier concentration and the volume added to each sample be constant throughout a single experiment.

3. Wet Ashing.

The samples in the digestion beakers are heated on a hot plate until fumes of sulphuric acid are observed. Fuming is continued for five minutes. The charred material, which appears even in the standard due to the destruction of the tartrate in the carrier, is eliminated by the dropwise addition of 16 N nitric acid, or 100 vol hydrogen peroxide, and then the samples are fumed for a further three minutes to eliminate the

excess oxidising agent. The solutions should now be pale yellow in colour. Sodium nitrate may be added in small portions up to a total addition of 0.5 g instead of peroxide or nitric acid. In this case the subsequent addition of 0.3 g of sodium sulphite ensures the reduction of excess nitrate. When digestion is complete, the digests are allowed to cool, and diluted with 5 ml of water. Usually this results in the precipitation of hydrolysis products of antimony, which are re-dissolved by the addition of 1 ml 11 N hydrochloric acid. The diluted solutions ar transferred with washings to 50 ml centrifuge tubes, and the volume made up to 25 to 30 ml.

4. Precipitation of antimony with chromous chloride.

The solutions are heated on a boiling water bath for three minutes, and 3 ml chromous chloride reagent are added. Heating is continued for further five minutes to coagulate the precipitate. This is spun down and the supernatant discarded. The precipitate is washed twice with water, taking care that its bulk is completely broken up with each wash, to reduce the interfering activity carried over to the next step of the separation. The failure of early trials of the method is attributed to this effect. Table B.7. shows the activities measured with an end window Geiger counter (beta activities) in comparison to the activities obtained using a sodium iodide crystal detector with pulse analysis set to count over the 0.57 MeV photopeak of Sb^{122} (gamma activities) for a number of samples and a standard after complete chemical separation. I: the isolated material is radiochemically pure the ratio of beta activity to gamma activity is the same for all of the sources. From the Table i' is seen that all but one pair of samples give significantly different values for this ratio. The half-lives of the samples with excess beta

activity, estimated by Geiger counting, are greater than that expected for radioantimony. The gamma spectra over the range 0.1 to 1.5 MeV do not show the presence of any gamma rays other than those of Sb^{122}. The inference is that the contaminant is a pure beta emitting isotope with a half life greater than 10 days. The most probable contaminant is P^{32} with a half life of 15 days. After allowing about 9 days for the antimony activity to decay by a factor of eight the contaminated sources are counted using an end window counter, with aluminium filters of different thickness interposed between the source and the detector. Plotting the count rate obtained with a filter of given thickness, expressed as a percentage of the count rate obtained with no filter present (% age transmission) against the thickness of the filter in mg/cm^2 gives a curve for each of the contaminated samples which is identical to that obtained with an authentic specimen of P^{32}.

Conclusive proof of the nature of the contamination is derived from the method of its elimination. The standard method of eliminating P^{32} in activation analysis is the precipitation of phosphate, for example with ferric iron or calcium. This type of precipitation is usually carried out in an alkaline medium, because phosphates in general are soluble in acid solution. This approach is inadmissible in the case of antimony because of the loss of antimony in an alkaline medium. Qualitative analysis, however, regularly applies a precipitation of phosphate carried out in an acid medium, using zirconyl salts (160). Precipitation of phosphate as phospho-molybdate is unsuccessful as it is impossible to achieve a suitable acid concentration without loss of antimony. This objection does not apply to the zirconyl phosphate method although the acidity of the solution does have to be reduced after the digestion step.

To eliminate phosphate, the digested sample is transferred to a centrifuge tube and diluted to 20 ml as before. Five ml saturated ammonium chloride is added, followed by 0.1 ml 10% (w/v) ammonium dihydrogen orthophosphate solution, and 0.25 ml 10 N sodium hydroxide. The addition of 0.2 ml 10% (w/v) zirconyl chloride in 0.5 N hydrochloric acid, followed by digestion of the precipitate on a boiling water bath for 5 minutes serves to precipitate the phosphate quantitatively. The precipitate is spun down and filtered off, and the supernatant treated with chromous chloride in the usual way to precipitate antimony. The remainder of the separation is the same as in the absence of phosphate precipitation.

Table B.8 shows the results for analysis of a variety of materials including teeth, where phosphorus in the form of phosphate is a major component of the sample matrix. It is seen that excellent agreement is obtained between results from comparison of the beta activity of sample and standard, and results obtained by comparing gamma activity, regardless of whether phosphate is specifically eliminated by precipitation, or the antimony precipitates are decontaminated by thorough washing.

5. Solution of Antimony Precipitate, and precipitation of Arsenic.

One ml arsenic carrier (equivalent to 10 mg As) and 6 ml 11 N hydrochloric acid are added to the washed antimony precipitate. The precipitate is dispersed in the solution as completely as possible by mechanical agitation, and following the addition of 0.35 ml 100 volume hydrogen peroxide the solution is agitated from time to time until the antimony has dissolved completely (about 5 minutes).

Immediately 2 ml of a saturated solution of ammonium hypophosphite

(approximately 7 M) is added. The solution is heated on a boiling water bath, until a brown precipitate appears, and then for a further 20 minutes.

6. Precipitation of Copper, and Preparation of Sources for Counting.

The solution from the previous step is removed from the water bath, cooled, and diluted to 20 or 30 ml. The addition of 0.25 ml of copper carrier is followed by the addition of 0.5 ml of 10% potassium ferricyanide. The excess ferricyanide is precipitated by 0.5 ml of 10% cobaltous chloride. The combined precipitates from this and the previou step are spun down, and the supernatant filtered off into a clean centrifuge tube. Antimony is precipitated once more by treatment with chromous chloride exactly as before. The precipitate is again washed thoroughly with water and then with acetone. The separated antimony is transferred to a weighed aluminium planchet as a suspension in acetone, dried under an infra-red lamp, and weighed to determine the chemical yield.

7. Counting and Calculation of Results.

The samples and standard activities may be determined either using a Geiger end window counter, or a thallium activated sodium iodide scintillation detector. The former method, though more sensitive due to the lower background count obtained, does not discriminate against possible contamination of the isolated material by bismuth. However, the standard precaution of repeating the counting procedure after about 3 day and checking that the decay of isolated activity is in agreement with the known half-life of Sb^{122} reveals the presence of any contaminating activity.

In calculating results for a large number of samples the following approach has a number of advantages. The count rates for the samples and standard are corrected for background and detector dead time. The activity of each source is then re-calculated to an arbitrarily selected chemical yield, expressed as mg Sb recovered. This has the effect of correcting all the activities to values which can be compared directly without any need to know exactly how much antimony was added initially as carrier or even calculating yields on a percentage basis. The standard activity is expressed in the form of a "count rate" for the analysis (C.R.) which is the specific activity generated in the standard for the irradiation conditions of a particular analysis. The units are counts/unit time /mg antimony, making allowance for the fact that the standard activity measured is that due to an aliquot of the amount of material irradiated as the standard.

It follows that for a given sample, the antimony content (p.p.m.) is given by:-

$$\text{Sb content p.p.m.} = \frac{\text{sample activity(counts/unit time-same units as for C.R.)} \times 10^6}{\text{sample weight (mg)} \times \text{C.R.}}$$

No account is taken of the decay of samples and standard during the period during which the activities are measured, because normally this time is short compared with the half life of the activity measured. In the case of antimony, since two active species are produced in the irradiation the decay curve can only be derived approximately by a straight line plot of log (activity) against time. The use of such a curve to estimate correction factors for the measured activities is less and less valid, as the time between the end of irradiation and counting

and the contribution of the longer lived Sb^{124} to the total activity increase. An accurate correction may be applied by following the decay of the standard and the samples over a fairly long period, constructing accurate decay curves, and correcting the measured activities by reference to these curves, or alternatively to the decay curve of the standard sample.

(c) Evaluation of the Method.

The specificity of the analysis is shown by tracer experiments with copper, arsenic, mercury, and Bismuth (this last in the form of a solution of Pb^{210}, the parent of Bi^{210}). All of these are eliminated except Bi. Decay curves obtained from the analysis of several samples under experimental conditions show half-lives essentially the same as that of Sb^{122}, with both Geiger and scintillation detectors (figure B.4.). The decay curves are not straight line plots due to the presence of the long lived Sb^{124} which cannot be separated from Sb^{122} even by gamma spectrometry.

Quantitatively, the proposed method is discussed under two headings. Trials with a solution of Sb^{124} show that equilibration between active material and carrier is complete, and demonstrate where losses of antimony occur during the separation. Excellent agreement is obtained between yields calculated gravimetrically comparing the weight of material recovered to the amount of carrier antimony added, and radiochemical yields based on a comparison of antimony activity recovered compared with activity added initially. Both methods in a series of trials show that yields of from 70 to 80% are obtained. Up to the completion of the first chromous chloride precipitation, 95% of the

initial amount of antimony remains in the system. About 10% is lost during the solution of the precipitated antimony, and 1 to 2% is lost with the discarded precipitate of arsenic and copper ferricyanide. The remaining 3 to 13% is lost in the second precipitation of antimony using chromous chloride, and the transference of the final precipitate to planchets for counting. The reproducibility and accuracy of the analytical procedure are determined as follows. Reproducibility tests require the availability of material of proven homogeneity. Such a substance has been made available by Bowen (17) consisting of kale, grown, harvested, dried and homogenised under carefully controlled conditions. The resulting material provides a biological material of proven homogeneity, in a form readily handled by analysts, and suitable in particular for activation analysis without further treatment. A sample of the dry material is activated along with an antimony standard i the usual way. To avoid errors due to loss of activated material in the transference of small samples from irradiation containers to digestion beakers, samples for analysis are weighed out after irradiation. Five determinations give the following results (p.p.m. dry weight):- 0.0537, 0.0603, 0.0560, 0.0623, 0.0645, median 0.0603, mean 0.0593, and standard deviation 0.0049 (8%). Bowen has revealed specimen analysis figures for the dried kale (Table B.9) where no value is quoted for the antimony content, but in a recent private communication, he quotes a value of 0.08 $\pm$ 0.02 p.p.m. The accuracy of the method is demonstrated by the analysis of standard solutions of antimony, with concentrations in the range 0.25 to 0.00675 μg/ml. These solutions have been irradiated and analysed in the fashion described

above for urine or other liquid samples. As can be seen from Table B.1 the theoretical and observed values are in agreement within the 10% limit claimed for the analysis. Assuming that the manipulative part of the analysis can be achieved without error, and that the activation process is also free from error, as indicated by the analysis of the standard solutions quoted above, the measurements involved in the calculation of a result are the only contributors to the total error of the analysis. It is easy to control the weighing errors associated with the preparation of sample and standard for irradiation, and also those associated with the estimation of chemical recovery of sample and standa: The controlling factor in the uncertainty of the analysis is the statistical nature of the count rates obtained from sample and standard source, and in particular the relationship between these activities and the background of the detector used.

Typically an end window Geiger counter has a background of 12 c/m. If the minimum activity which can be detected with an accuracy within th claimed limits of 10% is set equal to twice this value, so that such a source would give a count rate of 24 c/m above background, then the sensitivity of the method would be $24 \div (60 \times 10^6)$ mg Sb, where the denominator is the net count rate for an antimony standard under experimental conditions in counts/minute/mg Sb, or 4×10^{-10} g Sb. Thi: sensitivity refers to an irradiation for three days at a thermal neutron flux of 1.2×10^{12} n/cm^2/sec, activities being measured with a Geiger counter. For scintillation detection the sensitivity is about a factor of ten lower, if the same criterion for the minimum detectable activity is used. It is theoretically possible to reduce the counting error as

required by increasing the time for which both the background and sample counts are made.

The method described provides an accurate means for estimating antimony, far more sensitive than any method of classical chemistry. It is possible for a single operator to accomplish the separation and counting of 50 samples in two working days, with a third required for weighing out the samples and standard before the irradiation period. The method is not at all demanding technically and successful analyses can be achieved by personel who have had no previous experience of the technique. The final method fulfils the requirements laid down earlier.

B.3. Determination of Iodine in Biological Material with Special Reference to the Estimation of Protein Bound Iodine in Human Serum.

B.3.1. The Requirements for the Method.

The importance of thyroid function in controlling the basic metabolic processes is well known. Tata (124) reviews current theories on the mechanism of the control process. Basically the thyroid exercises its function by means of the hormones it produces. The significant feature of these hormones is the presence of iodine in the molecule, covalently bonded to aromatic residues. In clinical medicine the correct function of the thyroid can be examined in one of two ways. Iodine labelled with radioactivity can be given to the patient so that its localisation in the patients thyroid, and distribution through the body when incorporated into hormone molecules may be readily observed. Normal health and abnormal states are differentiated by the pattern of tracer uptake and elimination they produce. The hormone level in the blood of the patient is also an indication of the state of the thyroid. Here the determination is based on the fact that the thyroid hormone is virtually

the only protein containing bound iodine. Consequently, if blood, plasma or serum is treated in such a way as to remove all inorganic iodide, then the remaining iodine is a measure of the amount of thyroid hormone in the sample.

Both approaches are standard clinical tests for thyroid function. The latter approach, usually applied to the determination of protein bound iodine in serum, requires an extremely sensitive analytical technique, since iodine is to be determined at levels of around 2 to 10 μg%. The standard method for this analysis is based on that devised by Chaney (32). The plasma is freed from inorganic iodine by passing it through an anion exchange column in the chloride form. An aliquot of the eluate is digested with a mixture of permanganate, and sulphuric acid,or dichromate and sulphuric acid, by alkaline fusion (6) or more recently with chloric acid (169). In all these, the protein bound iodine is converted into iodate. Normally the iodate is reduced to iodine by a suitable reducing agent, such as phosphorous acid and distilled off. The iodine content of the distillate is then evaluated by a sensitive colorimetric method. The most common methods involve the measurement of the catalytic effect of iodide on the reduction of cerous sulphate by arsenious acid (32), the starch iodine chromogen (58) and the colour produced by elemental iodine dissolved in an organic solvent such as carbon tetrachloride (126). Raben (111) describes a method for labelling the iodine in the distillate, by adding it in the form of iodate to a solution of iodide of known specific activity. The free iodine produced is extracted with carbon tetrachloride, and comparison of its specific activity with that of the original solution of iodide is a measure of the added iodate. A similar

procedure is described by Oxby and Dawson (103) which is claimed to have a sensitivity of 1 μg % iodine. A bibliography on the micro-determination of iodine is available, summarising these and other techniques omitting radioactivation analysis methods (33).

The nuclear properties of chlorine, bromine and iodine are suitable for activation analysis purposes, (Table B.11), and it is not surprising that methods for their determination should be available. Bowen (18) describes procedures suitable for the estimation of all three elements in plant material and in blood. Essentially the separations are based on the dissolution of the sample in the presence of the appropriate halide carrier. The halogen is distilled into caustic soda. Several solvent extraction steps are used to separate the desired element from the others. The isolated material is finally precipitated in the form of the silver halide. Zvysgina, and Moskovtseva (77) describe a similar procedure, for bromine and iodine, except that the distillation step is avoided. Chlorine is determined non-destructively, using gamma spectrometry.

Similar methods have been applied to the estimation of protein bound iodine. In addition, several methods are described which make use of the properties of serum, and attempt to overcome the problems peculiar to the analysis of this material. The most significant of these is the decontamination of the iodine activity from interfering Cl^{38} activity, where the chlorine activity is of the order of 10^5 times that of I^{128} in the activated serum. American workers, such as Manney et al (90), and Smith et al (141), attempt to solve the problem by a preliminary removal of inorganic chloride along with inorganic iodide, using a weakly basic anion exchange resin in the hydroxide and acetate form respectively.

In both cases the interference is reduced, but not eliminated, and the analysis requires further decontamination from chlorine. The latter workers achieve this by a gamma spectrometric technique, and the former either by a fairly rigorous separation similar to that of Bowen, or by an instrumental technique similar in principal to that of Manney et al.

A French group, under Kellershohn and Comar (72) approach the problem in two ways. First they find it is possible to use activation analysis merely to replace the ceric-arsenite estimation in the usual micro-analytical method, i.e. to carry out the chemical separation before activation. They also apply the same separation after activation. The pre-irradiation application leaves the analysis as a whole liable to error by contamination by iodine from reagents, and the application of the chemical separation after irradiation requires time, which, because of the short half-life of the iodine activity, greatly reduces the sensitivity of the method. These workers have also examined the possibility of using ion exchange techniques to eliminate the bulk of the interfering elements from the serum before the material is activated. Lenihan (82) mentions the successful application by these workers of an activation analysis scheme, where after activation, dialysis, ashing, and multiple distillation steps the iodine is separated from the samples in a form suitable for counting.

It is clear that an activation analysis method for iodine is possible, indeed several methods exist, but what is required is some knowledge of the minimum acceptable processing of a sample after irradiation, which permits the isolation of the activated iodine in a suitably pure form for counting. It would be preferable if the detector were simply a Geiger counter, due to the greater simplicity, and sensitivity of this form of

detector, in comparison to a scintillation detector. Although it is standard practice to eliminate inorganic iodide from serum samples by ion exchange, it is felt that more extensive treatment of this nature is not worth while. According to the American workers (see above) the iodine activity still requires further purification by chemical methods despite the ion exchange step. If the chemical method is sufficiently stringent, there is no need for the ion exchange separation of contaminants other than inorganic iodide.

B.3.2. Radiochemical Separation.

For the activation analysis of serum for protein bound iodine, the first requirement seems to be for a suitable technique to destroy the protein, and leave the iodine in an inorganic form, without loss. This can be accomplished by the usual wet ashing techniques, provided the conditions are such that iodine is quantitatively oxidised to a non-volatile state, such as iodate. Alkaline fusion techniques can be neglected, on the grounds of their manipulative difficulty. From the survey of existing methods given above, the possibilities are acid permanganate, or dichromate oxidation, usually carried out under reflux, or chloric acid oxidation. In a series of trials chloric acid oxidation according to the method of Zak (169) is found to retain only 50% of the iodine added in the form of L-thyroxine. Of the other two methods, the permanganate technique is the more promising, due to its greater oxidising power. In this case, the iodine present is oxidised to iodate Subsequent reduction of the iodate to iodine can be followed by the distillation of iodine, which greatly assists the decontamination of the isolated iodine activity. The reducing agent used should be strong

enough to reduce iodate to iodine, without continuing the reduction to iodide. If dichromate is used in the digestion step, phosphorous acid can be used as the reducing agent (18). Oxalic acid is more often used when the first step has been carried out with acid permanganate (33). Simple qualitative tests confirm that hot oxalic acid will not only reduce iodate to iodine, without further obvious reduction but also the manganese dioxide formed from the decomposition of the permanganate in the digestion step, to manganous ions. Other reducing agents such as hypophosphite, ferrous iron, stannous chloride, and sodium arsenite, are all found to be strong enough to reduce iodate to iodide. It is impossible with these reagents to ensure that a large proportion of the iodate adopts the volatile zero valent state.

The combined distillation digestion apparatus, made entirely of glass, is substantially the same as that used by Bowen (18). The distillation head is fitted not only with a funnel for the introduction of liquid reagents, but also a connection for the passage of air through the solution to assist the completion of distillation. In the original design the flask is of 50 ml capacity, with a neck about 10 cm long. In view of the violence of the reaction, it is found to be more acceptable to use a flask of the same capacity, but with a longer (about 15 cm) and wider (B.19) neck. The volumes of permanganate solution and sulphuric acid used are determined by the nature and amount of material to be digested. It is found that the addition of 10 ml 36 N sulphuric acid to 10 ml 2N potassium permanganate (64 g potassium permanganate per litre of solution) is sufficient to destroy completely up to 0.1 g of dried biological material. The manganese dioxide and the iodate carrier equivalent to 20 mg iodine are completely reduced by the further addition of 6 ml saturated oxalic acid

solution. The products of distillation are conveniently trapped in dilute (5 N) sodium hydroxide.

Experiments with I^{131} tracer and inactive iodide both show that 90 - 95% of the iodine present initially is recovered from the combined digestion and distillation step. Comparing the stages in the process at which chlorine, bromine, and iodine appear in the distillate, using the appropriate radioactive tracers (I^{131}, Br^{82}, Cl^{36}) it is shown that chlorine is evolved in the presence of manganese dioxide almost completely (90%), and no further chlorine is detected in the distillate collected after the addition of oxalic acid. No iodine activity appears in the distillate, regardless of whether the iodine carrier is in the form of iodide or iodate, until after the addition of oxalic acid. The bromine activity is found to appear partly in the presence of manganese dioxide (60%), and the remainder on addition of oxalic acid, along with the iodine activity. These observations are entirely consistent with the known properties of the halogens. Hence it is desirable to discard the distillate collected during the digestion of the sample and to retain for further processing only the activity collected after the addition of oxalic acid to the digest. This eliminates a large proportion of the interfering chlorine activity.

Although the distillation step can be reasonably selective, further purification is required. Since it is more convenient to carry out the assay of activity and the chemical yield determination on a solid sample, it is acceptable to precipitate the iodine from the distillate. Usually iodine is precipitated as silver iodide, and if the precipitation is carried out in an ammoniacal solution, considerable decontamination from

chlorine can be achieved. The alkalinity of the solution must be carefully controlled. Much simpler is the use of palladous chloride. Addition of this reagent to a slightly acid iodide solution results in the quantitative precipitation of palladous iodide, provided the cation is present in excess. It is found that this step can be applied after the distillation, merely by rendering the distillate just acid to bromothymol blue with sulphuric or hydrochloric acid, and adding 3 ml of a 1% solution of palladous chloride in 1 N hydrochloric acid. Sodium arsenite is added to the caustic soda in the solution used to collect the evolved iodine, to ensure that all of the latter is reduced to iodide. Tracer experiments show that the palladous chloride precipitation step is 95% efficient, and that chloride and bromide are not co-precipitated. Further it is shown that chloride and bromide carriers can be added, to act as hold-back carriers and minimise the contamination of the precipitate by adsorbed interfering ions. Finally, the palladous iodide precipitate can be dissolved in 1 - 2 ml concentrated ammonia solution, and re-precipitated by the addition of a small excess of palladous chloride, after rendering the solution just acid. The recovery from a distillation and palladous chloride precipitation step is found to be 80 - 90% determined by both gravimetric and tracer methods.

The combined distillation precipitation separation applied to dried serum isolates material whose half-life (25 and 26 min in two trials) agrees with the literature value of 25 min for I^{128}(2).

The attempted analysis of a standard serum gives the decay curve shown in figure B.5. and the gamma spectrum shown in B.6. In these

two figures it is seen that the nuclear characteristics of the material isolated from the serum are essentially identical tothose of material isolated from ammonium iodide, and di-iodotyrosine activated along with the serum. It is particularly difficult to estimate the purity of isolated iodine. Decay curve analysis is not fully satisfactory for this purpose because the likely contaminants are bromine and chlorine which give activation products whose half-lives are very similar to that of I^{128} (Table B.11). If the decay of activated samples is followed long enough, even if every precaution is taken to ensure that only iodine is present before activation the decay curve reveals the presence of traces of a very long lived activity. Although present at a level which is too low for its half life to be estimated with any certainty, and certainly too low for its gamma emissions to be characterised, this appears to have a half-life of several days. There is strong presumptive evidence that this contaminant is I^{126}, half-life 13.3 days (2).

A sample of ammonium iodide is activated for three days at a thermal neutron flux of 1.2×10^{12} thermal neutrons per cm^2 per sec. On return to the laboratory the activated material is dissolved in dilute sulphuric acid, in the presence of antimony carrier. The antimony is precipitated and purified, but the supernatant from the precipitation is filtered and the iodine extracted into chloroform after the addition of excess sodium nitrite solution. The iodine is back extracted into alkaline arsenite solution after washing the chloroform with water. The gamma spectrum of the final extract is shown in Figure B.7. and is identical with the published spectrum of I^{126} (2).

The spectrum of the original solution after the extraction of iodine is that of Br^{82} (Figure B.8.). It is scarcely surprising that the half-life of the activity in the final extract should be about 6 - 7 days when measured over a three day period just after extraction, due to contamination by bromine activity. After a delay of 14 days, to allow the bromine activity to decay re-evaluation of this half-life gives a value of 12.2 days. These results are strong evidence for the formation of I^{126} in the pile activation of iodine. It is not known whether the nuclide is the result of an (n, 2n) reaction due to fast neutrons in the flux of the pile, or a (γ,n) reaction. The evidence for the latter is that Smith (~~127~~ 137) has demonstrated the production of this activity in a sample of ammonium iodide exposed to the gamma radiation from a synchrotron. Mulvey et al (97) have proposed the use of bremsstrahlung radiation producing I^{126} as the basis of an activation analysis method for the estimation of iodine, which they have applied successfully to solutions containing microgram amounts of iodine, even in the presence of large amounts of sodium and chlorine.

The halogens are readily distinguishable by their gamma emissions (Table B.12) but the low abundance of the principal gamma ray of I^{128} (17%) and the relatively greater abundance of the 2.12 MeV beta particles (76%) mean that detection by gamma spectroscopy is less sensitive than Geiger counting. Consequently a source containing I^{128} may give a reasonable count rate with the Geiger counter, and yet the 0.46 MeV photopeak of I^{128} is not observed in the gamma spectrum. Gamma spectrometry cannot be applied as a test of radiochemical purity for I^{128} if the activity to be characterised is at or near the limit of

detection by Geiger counting, which is usually the case in the analysis of serum.

The simple two stage method outlined above gives satisfactory radiochemical purity within the limits of the usual tests. Using solutions of ammonium iodide or L-thyroxine it is possible to demonstrate a linear relationship between activity isolated and the iodine content of the solution. It is found that this can only be achieved if the standard samples are activated in the form of solutions. The alternative approach of mounting solid material on polythene sheets for ease of irradiation is impracticable due to the loss of iodine as the solutions containing the known amounts of iodine are evaporated to dryness.

Application of the method to serum of known iodine content gives disappointing results. Not only are the activation analysis results different from the results obtained by the usual chemical method, but it is found to be very difficult to obtain reproducible results, especially with serum with normal or lower than normal iodine content (Table B.13). At this stage 0.1 ml samples of serum are activated in polythene tubes, along with a standard solution of either L-thyroxine or ammonium iodide. The samples and standard are activated for 25 min at a thermal neutron flux of $1.0 \times 10^{12} n/cm^2/sec$, and processed with the addition of iodine carrier in the form of iodate. This form is chosen because it does not require the carrier to be oxidised to attain this valency with the risk of the loss of volatile iodine during the oxidation process. It does not matter if the active iodine is in the form of iodide and is volatilised. The small amount of material evolved is almost certainly adsorbed on to the apparatus surfaces and exchanges with the carrier when

the latter is distilled off. No difference is observed between results obtained when the samples and standard were removed from their irradiation containers before processing, and when processed along with the opened containers. The hazard of cross-contamination between samples is eliminated by sealing the tubes before irradiation. An additional precaution is to seal each tube in a polythene envelope and discard the latter after irradiation is complete.

In viewof the variable results obtained it is thought that chlorine activity may be coming through the separation in amounts sufficient to invalidate the analysis, although the photopeaks characteristic of Cl^{38} are never observed in the spectra of the isolated material. Due to the low activities iodine is not observable either.

In any case a solution of sodium chloride has been prepared containing 9.0 mg NaCl/ml, about the same concentration as in serum, activated and processed. If the separation is effective, no activity at all should appear in the final precipitate. Activity is in fact detected after the separation, but the extension of the method by the adoption of multiple palladous iodide precipitations, or the introduction of a solvent extraction step using sodium nitrite and chloroform, fails to eliminate the activity. Eventually it is shown by gamma spectrometry to be due to iodine present in the solid sodium chloride to the extent of approximately 0.1 p.p.m. or 0.01 µg iodine per ml of sodium chloride solution.

It is found that a large proportion of the Cl^{38} activity in the distillate is extracted into chloroform along with the iodine. However, the bulk of the interference is removed by washing the inorganic extract

with an aqueous acid solution of sodium chloride. The success of the wash in removing the interference from the organic layer is due to exchange between excess aqueous chloride and chlorine dissolved in the carbon tetrachloride, as well as the removal of residues of the aqueous layer from the previous extraction step.

The original method is based on the assumption that it is necessary to destroy the iodine containing molecules of the serum to release all of the activated iodine. Comar et al (36) have shown that after the activation of a solution of thyroxine at least 95% of the iodine activity can be retained on an anion exchange column, and hence activation strips the iodine atoms off the protein molecules and leaves them in the form of inorganic iodide. Comar and Le Poec (35) have determined iodine in a variety of biological fluids, including blood and serum, using activation followed by ion exchange alone to purify the iodine activity. The success of this method indicates that the digestion technique described above can be simplified. The omission of permanganate oxidation eliminat the hazardous addition of concentrated sulphuric acid to permanganate solution, and the use of iodate carrier permits the addition of oxalic acid to the sample and carrier mixture before the commencement of the distillation. This overcomes the problem of the evolution of large volumes of carbon dioxide and the risk of bumping which accompanies the oxalic acid reduction in the original process. Using oxalic acid alone in the distillation process does not allow the Cl^{38} activity to be distilled off. Hence it is necessary to insert a solvent extraction step between the distillation and the final precipitation of palladous iodide to attain the required degree of purity.

It is found to be better to use 0.5 ml samples of serum. The five fold increase in sample volume greatly aids the transfer of samples from the irradiation containers to digestion flasks. Standards were conveniently prepared by the irradiation of milligram amounts of L-thyroxine. The activated standard is transferred along with the open irradiation vial to an alkaline solution of sodium iodate to ensure solution of L-thyroxine. Acidification results in the formation of iodine from the added carrier and inorganic iodide produced in the activation process, and assists in the complete extraction of activated iodine from the standard container. The solution formed is diluted suitably, and an aliquot processed in the same way as the samples. The simplified method gives a linear relationship between iodine content and isolated activity when applied to solutions of L-thyroxine, but the results of its application to sera of known iodine content are again disappointing (Table B.14). Sample No. 11 gave sufficient isolated activity for gamma spectrometric confirmation of the value obtained by Geiger counting.

Two samples of dried kale analysed against an L-thyroxine standard, where the samples have been treated with the full digestion technique to dissolve them completely and the standard processed by the short form of the method gives results of 0.268 and 0.246 p.p.m. as against Bowen's value of 0.27 (Table B.9). The results of kale analysis, where the iodine content of the kale (largely inorganic), is measured against the iodine content of L-thyroxine, where the iodine is bound to a benzene ring as it is in the serum iodo-proteins, confirms the completeness of the Szilard-Chalmers process in the activation of these proteins for the

estimation of iodine. The failure of the proposed method when applied to serum is still unexplained. It is unlikely in view of the decay curve and gamma spectrometric studies already carried out that interference from Cl^{38} in the course of the chemical separation is the cause. The question remains as to the accuracy of the chemical results with which the activation results are compared. Analysis of standard sera such as those used in the calibration of the chemical method itself may supply the answer to this question.

B.3.3. Conclusions on this Investigation.

The activation analysis method for iodine described above seems to be suitable for the estimation of iodine in biological material, and its failure in the analysis of serum remains unexplained. Bearing in mind the fact that the activation of serum results in the release of the activated iodine in an inorganic form, an alternative procedure may use the equilibrium between aqueous iodide and a solution of iodine in an organic solvent such as carbon tetrachloride. Preliminary studies show that iodide labelled with I^{131} can be completely extracted from an aqueous solution by shaking it with a solution of iodine in a solvent immiscible with water. The application of this technique, once the problem has been overcome of ensuring that the activated serum iodine is in the lowest valency state, should provide the basis for a rapid and extremely selective separation. In view of the difficulty of characterising the isolated activity at levels acceptable for analytical purposes, the only approach to the development of any proposed method is one of trial and error. This requires the availability of sera whose protein bound iodine content is known with a high degree of certainty.

B.4. Multi-element Analysis by Complex Chemical Separation.

B.4.1. Introduction.

As mentioned earlier it is possible to achieve the analysis of a single sample for several elements at once, using a complex separation procedure. The usefulness of such a process depends on the activation characteristics of the elements to be determined, as well as on their chemical properties. Copper, zinc, and cadmium, are a group of elements whose activation characteristics are compatible. Vogel (159) describes an ion exchange separation suitable for the gravimetric or colorimetric analysis of mixtures of these elements, which can be modified to meet the requirements of a radiochemical separation.

B.4.2. Chemical Separation.

Previous work by Stojanovic (149) has demonstrated that separation can be effected on mixtures of copper, zinc, and cadmium, labelled with the appropriate tracers. Biological material is digested in nitric acid, and dissolved in a solution suitable for ion exchange by evaporation to dryness, followed by repeated evaporations in the presence of 11N hydrochloric acid. Finally the ashed sample together with the added copper, zinc and cadmium carriers is taken up in 0.5 N hydrochloric acid. This solution is passed down a column of Amberlite IRA-400 in the chloride form. Zinc and cadmium form complex chloro ions which are adsorbed on the resin. Copper does not and is eluted in part with the 20 ml of 0.5 N acid in which the samples were dissolved initially, and the remainder washed out with 25 ml of 0.12 N hydrochloric acid in 10% (w/v) sodium chloride solution. Copper is separated from the combined eluates by the method

of Smith and Nixon (99) after concentrating the solution by evaporation.

Zinc is eluted with 30 ml of 2N sodium hydroxide in 2% (w/v) sodium chloride. It is found that this fraction is usually contaminated with P^{32}. The latter is eliminated by making the solution just acid with sulphuric or hydrochloric acid, adding 0.5 ml 10% (w/v) ammonium dihydrogen orthophosphate solution, and precipitating zirconyl phosphate by the addition of 0.6 ml of a 10% (w/v) solution of zirconyl chloride in 1 N hydrochloric acid. The excess zirconyl chloride is eliminated by the addition of a further excess of orthophosphate after the initial precipitation is completed by heating. This prevents interference by the zirconyl ion in the precipitation of zinc as the quinaldate. The method of eliminating phosphate by a ferric hydroxide scavenge is found to be less satisfactory due to the incomplete elimination of the interference, and the loss of large amounts of zinc in the alkaline medium required. The zirconyl phosphate precipitate from the previous step is spun down and the supernatant filtered off. The solution is made just alkaline with caustic soda, and then just acid once more with acetic acid. Addition of excess of quinaldic acid to the hot solution results in the precipitation of zinc quinaldate. The precipitate is spun down, washed with water and acetone in the usual way, and transferred as a slurry in acetone to an aluminium planchet for weighing and counting.

Cadmium is eluted from the column with 50 ml of 1 M nitric acid. The mechanical problem of reducing the volume of solution containing the cadmium is solved by making the eluate alkaline with sodium hydroxide, heating to coagulate the cadmium hydroxide precipitate, and filtering it off. The washed precipitate is washed through the filter into a

centrifuge tube with 20 ml 0.5 M hydrochloric acid. Cadmium reineckate is precipitated from the solution by the addition of 5 ml of 5% (w/v) thiourea solution, followed by 8 ml of a saturated solution (room temperature) of ammonium reineckate in 1% (w/v) thiourea. The precipitate is washed with thiourea solution and alcohol, and transferred to an aluminium planchet, for weighing and counting as a slurry in alcohol.

B.4.3. Activation Properties of Copper, Zinc and Cadmium.

As shown in Table B.15 each of the three elements has several useful neutron capture products for the purposes of activation analysis. The only copper isotope applicable is Cu^{64}. In the case of zinc, Zn^{65} is too long lived for its apparently greater sensitivity to be available. If use is made of the most sensitive zinc isotope, Zn^{69}, then the optimum sensitivity is achieved after one hour's irradiation, which is unsuitable for the estimation of copper. On the other hand, if irradiation is carried out for twelve hours for the benefit of the sensitivity with which copper can be determined, then the samples become rather active for safe handling until they have decayed for about eight hours, so that Zn^{69} has decayed, although the less sensitive Zn^{69m} is now available. The metastable isotope gives an analytical sensitivity of one tenth of that available with Zn^{69}, but this is adequate for most purposes. The longest lived cadmium isotope is discarded for the same reasons as Zn^{65}. The remaining isotopes both of which decay to form indium daughter activities are useful. It is reasonable in an analysis for zinc and cadmium, omitting copper, to activate for 3 hours and to determine zinc by Zn^{69} and cadmium by Cd^{117}/In^{117}. The difficulty here is that the time available for processing is limited by the half lives of the sought for activities,

so that the number of samples analysed in a single run may be limited. In practice all three elements may be detected with reasonable sensitivity if activation is carried out for 3 days, and the activities sought are Cu^{64}, Zn^{69m}, and Cd^{115}/In^{115m}.

B.4.4. Evaluation of the Technique.

The method has only been investigated with a view to its application to the determination of all three elements. Samples are activated for three days along with metallic zinc, and cadmium oxide standards. At this stage there is no question as to the suitability of the proposed method for copper so this element is not discussed. Zinc analysis of teeth samples gives values of 165.0, 184.0, 199.0 and 292.0 p.p.m. in agreement with results obtained subsequently using a similar ion exchange separation using Zn^{69} activity (86). The purity of the isolated material is demonstrated by the half life values obtained, ranging from 14.4 to 13.6 hours indicating the successful elimination of phosphorous interference. The gamma spectrum of one of the final precipitates (Figure B.9) shows only photopeaks due to zinc isotopes. Liver samples in the same experiment satisfied the same tests for purity, although the lower phosphorous content of these samples makes them a less severe test of the method. The values 152.0, 161.0, and 169.0 p.p.m. dry weight agree with the range of values published by Tipton (154), maximum 279.0 p.p.m. and minimum 62.0 p.p.m., relative to dry weight of the tissue.

In the case of cadmium, the teeth samples are so low in this element that the isolated activities cannot be characterised, but the liver samples give values of 3.9, 4.1, 3.4, and 3.3 p.p.m., again inside Tipton's range of 1.9 to 33.4 p.p.m. dry weight. The decay of these samples is

complex because the indium daughter grows back into equilibrium after the completion of the separation, so that for analytical purposes it is necessary to allow 24 hours to elapse between the mounting of the sources and the measurement of their activities. However, it is found for the liver samples that the activity measured on a Geiger counter give a curve parallel to that obtained from the standard sample. The gamma spectra of the cadmium precipitates from the liver samples taken after equilibrium is reached between the cadmium and indium activities (Figure B.10) shows only photopeaks due to Cd^{115} and In^{115m}.

B.4.5. Summary.

For the group of elements copper zinc and cadmium, the ion exchange separation outlined above, coupled with the various possible isotopes provides a means of determining these elements in a variety of combinations with considerable sensitivity. Geiger-Muller counting permits the estimation of 0.1μg of cadmium, and zinc, after irradiation for three days at a thermal neutron flux of 10^{12} n/cm^2/sec. Using the short lived zinc isotope (Zn^{69}) Geiger counting will detect 0.01 μg with ease, but if cadmium is to be estimated simultaneously the sensitivity for this element will be decreased by about a factor of four to about 0.5 μg, and the sensitivity for copper is reduced even more drastically. Bowen and Cawse (21) have published a number of methods for activation analysis for different elements including copper and zinc, and it is a simple matter to determine zinc and copper simultaneously in a single sample by combining the precipitation separations of the two elements, at the point where copper is separated from zinc by thiocyanate precipitation. The precipitate, processed according to the method of Nixon and Smith, leads to a pure

copper activity. The supernatant can be processed in the usual way, giving the pure zinc activity. The ion exchange separation is simpler, faster, and certainly preferable when short lived activities are sought. It is essential for cadmium analyses.

B.5. Conclusions on the Development of New Activation Analysis Methods.

At the present stage in the development of activation analysis using thermal neutrons, new methods tend to be improvements on existing techniques rather than "new". New, faster, separation techniques permit the use of activities previously neglected because of their short half lives. The techniques adopted in radiochemical separations continue to be drawn from the field of classical analytical chemistry, and developments in activation analysis parallel those in the parent field. The standard tests for the specificity of activation analysis methods remain the determination of the physical constants of the isolated material involving the determination of half lives and gamma spectrometry. The problem of determining the quantitative accuracy of an activation analysis method remains exactly the same as in classical analysis, involving the preparation and analysis of accurate test materials.

C. SOME APPLICATIONS OF ACTIVATION ANALYSIS TO PROBLEMS IN THE FIELDS OF MEDICINE AND FORENSIC SCIENCE.

C.1. Activation Analysis applied to Clinical Studies of Wilson's Disease.

C.1.1. Introduction.

Wilson's disease is characterised by disfunction of the metabolic processes involved in the normal use of copper in humans. Normally the serum copper is almost entirely bound to protein in the form of ceruloplasmin. In the diseased state the individual is unable to form ceruloplasmin to the full extent, with the result that not only is the ceruloplasmin level in the serum lower than normal, but in addition, copper is deposited in the liver and the cornea. The latter effect gives rise to the appearance known as ' Kayser-Fleischer rings ', characteristic of the disease. The terminal nature of the disease is largely due to hepatic failure due to the deposition of copper in abnormal amounts in this organ. In addition to liver disfunction the disease often produces nervous disorders of varying degrees of severity. It is not understood whether the relationship between abnormal copper metabolism and the hepatic involvement and renal lesions which are the justification for the alternative name for the condition, hepato-lenticular degeration (HLD), is one of cause, or effect. Attempts to produce lesions, in experimental animals, similar to those found in Wilson's disease by the administration of large amounts of copper in a variety of forms have been completely unsuccessful. The treatment for the condition consists of minimising the dietary copper intake of those who suffer from it, and the use of complexing agents such as British anti-Lewisite (BAL), or

penicillamine to assist the excretion of ingested copper. One of the most curious aspects of the disease is that the inability to form ceruloplasmin effectively is a heritable trait, so that the condition tends to run in families.

The diagnosis of the condition depends on the observation of the patients condition for symptoms of neurological involvement, the 'Kayser-Fleischer rings', clinical evidence as to liver function, and the determination of copper levels in serum, but more significantly in the liver. In serum examination the significant feature is the ceruloplasmin content, and not the total copper content. In some cases of Wilson's disease the non-protein bound copper in the serum is present in greater amounts than in normal patients, compensating for the reduced ceruloplasmin content. A valuable synopsis of the nature and treatment of this condition is available in 'Metal-Binding in Medicine' (125).

Activation analysis can be used in the diagnosis of the condition in the estimation of tissue copper contents, and the analysis of serum. It is of value to follow the course of treatment with complexing agents by examining the output of copper in the urine as treatment proceeds. This also can be done by activation analysis.

C.1.2. The Method of Analysis Used.

Copper is readily estimated by activation analysis using thermal neutron activation to produce the isotope Cu^{64}. If activation is carried out for 24 hours at a thermal neutron flux of $10^{12} n/cm^2/sec$, then chemical separation by the method of Nixon and Smith (99), and activity estimation using an end window Geiger counter permits the estimation of down to 10^{-9}g of copper with an accuracy of better than

10% with ordinary care. The chemical separation used is a precipitation technique. The samples are dissolved in nitric acid, and finally taken up in 0.25 N nitric acid. Copper is separated from the bulk of interfering activity as cuprous thiocyanate by the addition of sodium sulphite and potassium thiocyanate. The precipitate is washed, and dissolved in nitric acid. Residual contaminants are removed by precipitation of ferric hydroxide, using ammonia to retain copper in solution. Copper is precipitated from the acidified supernatant as the thiocyanate as before. Finally the copper is redissolved in nitric acid, the pH of the solution carefully adjusted and copper quinaldate precipitated for weighing and counting. The method is essentially a modification of that of Bowen (21).

When the method was adopted originally its accuracy and specificity were thoroughly investigated by the usual means. Further tests are applied in the present investigation.

C.1.3. Analysis of Serum.

Under the conditions of irradiation used blood serum and plasma is denatured to form a jelly-like mass which is impossible to remove quantitatively from the silica containers used in the irradiation of liquid samples. Drying the serum over silica gel under a reduced pressure of 1 mm of mercury or less produces a powder, which can be packed in polythene tubes or aluminium foil. Transfer after irradiation to the vessel used for digestion of the sample is then simple. Fortunately, it appears from the results obtained, that the water content of serum is relatively constant, and conversion from the analytical values obtained as p.p.m. dry weight to μg/ml is readily

achieved by multiplication by a factor of 0.0718 derived from the water content and density of serum as quoted by Bowen (19). Practical proof of the accuracy of the analysis is derived by the analysis of eleven samples of Bowen's dried kale. The results ranging from 4.00 to 5.01 p.p.m., mean 4.41 p.p.m., median 4.30 p.p.m., with a standard deviation of 0.34 (7.7%), show a reasonably narrow distribution around the provisional 'true' value of 4.4 p.p.m. (17). A better test still is the fact that six identical aliquots of copper activity processed by the proposed chemical separation in the presence of inactive dried serum give recovered activities, corrected for chemical recovery, of 913.0, 1011.0, 1031.0, 996.0, 929.0 and 925.0 counts/min, with a mean value of 968.0 counts/min, and a standard deviation of 12.9 counts/min, as against the activity of 960.0 counts/min of a seventh identical aliquot.

This experiment confirms the quantitative nature of the separation and the associated gravimetric recovery correction.

As a final test of the accuracy and reproducibility of the method, copper sulphate solution has been added to aliquots of a large volume of serum, and the original serum and each of two copper rich samples analysed in duplicate. The results shown in Table C.1. are self consistant within 10%, and the duplicate analyses indicate reproducibility in the same range.

The serum samples analysed in the investigation of Wilson's disease are all analysed in duplicate, and in all cases the replicate analyses are within 10%. The serum analyses fall into two parts. First 10 normal serum samples taken from orthopaedic patients in the

Royal Infirmary, Glasgow, have been analysed. The results listed below are in each case the average of duplicate estimations.

Normal Serum Copper Content (μg/ml).

0.86, 0.93, 0.98, 0.98, 1.03, 1.06, 1.30, 1.32, 1.36, 1.50.

The median value is 1.06 μg/ml, the mean 1.13 μg/ml, and the standard deviation 0.22 μg/ml (20% of the mean value). The mean value for total serum copper given above is in excellent agreement with the value of 1.14 μg/ml of Shields et al (125) and also the value accepted by Bowen (19). Shields' value has been determined by a colorimetric estimation with diethyldithiocarbamate. For comparison a further ten sera have been pooled and the combined material analysed in duplicate to give a mean value of 1.01 μg/ml, within the normal range. Finally, sera from three patients being treated with D-penicillamine, but not for Wilson's disease, have been analysed. The results, 0.85, 0.94, and 1.01 μg/ml are all lower than the normal mean or median, but within the normal range. This indicates that the treatment with the chelating agent of these patients, whose copper metabolism is normal is not depleting their serum copper level unduly. In these patients presumably the bulk of the serum copper is in the form of ceruloplasmin and not affected by the complexing agent.

In its application to Wilson's disease, activation analysis of serum is applied as a screening test to the members of the families of patients in which the disease has been diagnosed. The serum copper level alone, is, as explained above an unreliable test for Wilson's disease, unless confirmed by an immunological analysis specific for

ceruloplasmin. The serum copper levels for three families are shown in Table C.2. The first member in each family is the patient in which Wilson's disease has been diagnosed. Subsequent investigation by clinical tests has cast some doubt on the original diagnosis of S.W., accepted initially due to the previous death of a child in the same family attributed to Wilson's disease, and the presence of Kayser-Fleischer rings.

In the case of W.S., only his father shows a low serum copper content. This finding has been confirmed by immunological investigations. It is thought that the father is a sub-clinical case of Wilson's disease, and that W.S., inherited the condition from his father. In the family of T.F., one other member of the family, his sister, I.F., shows a very low serum copper level. Further clinical investigation has confirmed the presence of Wilson's disease in this patient, who is currently undergoing treatment with penicillamine. In the family of S.W., no other member of the family shows abnormal serum copper levels, and his own serum copper level is not as low as in any of the other cases of Wilson's disease suspected or confirmed.

The contribution of activation analysis to the determination of these serum copper levels, is to provide a method of proven specificity and accuracy, giving analytical results free from the doubts associated with the technical difficulties accompanying analysis by the more usual colorimetric methods.

C.1.4. Analysis of Urine.

The normal variation in the composition of urine not only from

individual to individual, but also with time, prohibits the analysis of dried urine samples. The analysis of whole urine is readily accomplished by weighing 0.1 g aliquots into silica tubes, which are heat sealed to prevent loss of material during activation, and in the handling of the irradiation container. Quantitative transfer of the activated urine from the irradiation container is ensured by rinsing the container thoroughly with an acid solution of the required volume of copper carrier in dilute (1 N) nitric acid. All washings and the excess wash liquid are transferred to the digestion beaker, so that the sample is digested in the presence of the correct amount of added carrier copper. The digestion and processing of the transferred sample is completed in the same way as described previously for serum samples.

The accuracy and reproducibility of the analysis is demonstrated by the preparation of copper rich samples by the addition of copper sulphate to aliquots of urine. The results of duplicate analyses of the original urine and two copper rich solutions prepared from it are shown in Table C.3. The reproducibility of the duplicate analyses, and the agreement between the calculated and the observed copper contents of the enriched urines, are both within the 10% tolerance claimed for the method. A criticism which might be levelled at the analysis is that very small samples are used, which could lead to erroneous results if the original urine sample is not homogeneous. Very often a precipitate of insoluble ureates forms in urine which is stored for some time before it is analysed. Such a urine sample has

been sampled (a) without disturbing the precipitated material, (b) after suspending the precipitated material by vigorous shaking, and (c) in such a manner that the sampled material is almost entirely made up of precipitated material. The results of the analyses of these samples are as follows:-

(a) Urine before shaking 0.960, 0.915 μg/ml

(b) Urine after shaking 0.996, 1.03 μg/ml

(c) Precipitated material 2.60, 2.26 μg/ml

The difference between the values before and after shaking does not exceed the accuracy claimed for the analysis. Considering the relative volumes of the precipitated material and the urine in which it is formed the copper content of the solid, though significant in terms of the units in which it is measured, is negligible in terms of the total copper content of the original urine sample.

In a study of Wilson's disease the analysis of urine samples is not a diagnostic aid,but a means of following the progress of treatment For comparison purposes a series of urine samples (24 hour collections) from orthopaedic patients was analysed (Table C.4.). The results are expressed both in terms of copper content in μg/ml and mg of copper excreted per diem. The copper content of the urines ranges from 0.040 to 0.223 μg/ml, with a median value of 0.079 μg/ml, a mean of 0.097 μg/ml and a standard deviation of 0.057 μg/ml (59% of the mean value). On a per diem basis the values range from 0.03 to 0.33 mg, with a mean and median value of 0.12 mg, and a standard deviation of 0.08 mg (67% of the mean value). The adjustment of copper content to amount excreted per day broadens the distribution of values as measured which are considered as normal, as measured by the standard

deviation expressed as a percentage of the mean value. Reference to the table shows that this correction alters the position of the samples in a series in which the results are set out in order of magnitude. Hence it is better to express the results of urine analysis in terms of the daily extretion. This approach at least eliminates the effect of the fluid balance mechanism in altering the concentration of the urine over a period of time.

In Table C.5. are shown the results of urine analyses performed on samples from patients with normal copper metabolism, but under treatment with penicillamine. The values are only available in terms of concentrations, and oover a much wider range than the normal urine samples. The median value for these patients is 0.210 μg/ml, the mean 0.238 μg/ml and the standard deviation 0.244 μg/ml. It is seen that the mean value minus one standard deviation gives a value of less than zero, which is meaningless in this context. The reason is that the calculation of a standard deviation implies that the results so treated lie in a normal distribution. Then the mean value plus or minus one or two standard deviations will lie within the range of values obtained on either side of the mean. The anomaly arises in the present case because the results lie on a distribution biased in favour of results lower than the mean value, as shown by the fact that the median value is less than the mean value. Unfortunately, no information is readily available as to the relationship between the time when the samples were taken and the regimen of penicillamine treatment applied to these patients. It is suggested that the great variation in the urine content of copper is due to large amounts of copper being excreted at the commencement

of treatment, leaving ceruloplasmin (bound) copper unaffected. Later the only copper complexed and excreted due to the action of the chelating agent is the minute amount absorbed from the diet. This proposal is not only in agreement with the serum copper results for these patients, but is substantially the conclusion reached by Walshe (125).

A sample of urine from a case suspected on symptomatic grounds of being Wilson's disease shows that the level of copper excretion is 0.058 mg/diem, or 0.064 μg/ml, well within the normal range. As with serum copper estimation, this test is of little value on its own.

In the cases of Wilson's disease, confirmed by other tests, the urine copper level is determined at intervals over a period of time to observe the change in the amount of copper excreted as treatment proceeds. The values obtained for each of the four cases studied are listed in Table C.6. It is seen that in almost all instances the daily copper excretion in cases of Wilson's disease exceeds the normal value from the orthopaedic patients (Table C.4.). The lower end of the range of values encountered in the treatment of Wilson's disease overlaps the range obtained in the urine copper content from patients with normal copper metabolism, treated with penicillamine. (Table C.5.) In the case of W.S., the daily copper excretion in the urine is shown in Fig. C.1. Also shown are the normal daily copper excretion (mean ± one standard deviation) calculated from the results in Table C.4., and the time at which treatment with the chelating agent commenced. Although the values fluctuate the overall effect is clear. At the commencement of treatment large amounts of copper are excreted, but excretion diminishes as treatment proceeds. This is in agreement with

the findings of Scheinberg and Sternlieb (125). Broad agreement is obtained with urine estimations carried out by the usual colorimetric methods, although some samples give distinctly different results in the two methods, repeated estimations by both methods having eliminated errors other than systematic errors. The colorimetric method gives high values. It is thought that this is due to interference by another element present in the urine in unusually large amounts due to the action of the chelating agent. Butler and Forbes (29) have pointed out that the use of colorimetry in this type of analysis is not without difficulties of this kind.

C.1.5. Analysis of Dry Tissues.

Material such as liver, or hair and nail, is dealt with in the dry state, when the chemical treatment involved is exactly the same as for serum (see above). In this study such analyses applied to liver from a case of Wilson's disease (W.S.) show a copper content of 145.0 p.p.m. (dry weight), about seven times the normal value. A case suspected of being Wilson's disease is shown to be due to cirrhosis by the liver copper content of 3.59 p.p.m. In a confirmed case of Wilson's disease (W.S.) it is found that the spleen copper content (7.09 p.p.m.) is not significantly different from the normal value. All these determinations have been carried out at least in duplicate, and results of the replicate analyses all lie within the 10% reproducibility claimed for the method. For example, the liver copper content in the Wilson's disease case is 144.0 and 146.0 p.p.m. in analyses each on 2.0 mg of dry tissue.

Analyses of the hair and nails of three patients with Wilson's

disease (T.F., I.F., and S.W.,), of the other members of S.W's family, and of the case of cirrhosis of the liver, show results entirely consistent with the copper content of normal hair and nail. (Table. C.7.)

C.1.6. Summary.

This illustrates an application of activation analysis to clinical medicine. The advantages of the technique, sensitivity, accuracy, and simplicity of application to a range of materials, commend the method for routine use in situations where accurate elemental analyses are required. To accomplish the same analyses by colorimetry would require quite different methods for urine, serum, and whole tissue. In addition the specificity of the activation analyses is clearly demonstrated by half life determinations and gamma spectrometry. There is no check on the selectivity of the colorimetric methods apart from the initial calibration experiments, and investigation of the tolerance of the method for specific interfering elements.

C.2. Activation Analysis and the Toxicology of Mercury.

C.2.1. Introduction.

The toxicity of mercury and its compounds has been long established. Inhalation of the vapour, ingestion of soluble compounds, and the absorption of the metal through the skin are all accepted means of intoxication. Ordinary chemical analyses are rather insensitive when applied to the investigation of sub-lethal quantities of the element except in special circumstances. A suitable activation analysis technique permits a study of the

occurrence of the element in both normal and exposed individuals.

C.2.2. Activation Analysis of Mercury.

Thermal neutron activation of mercury produces three isotopes applicable in an activation analysis determination of this element, Hg^{197m}, Hg^{197}, and Hg^{203}, with half-lives of 24 hrs, 65 hrs, and 47 days respectively. The original method of Smith (138) determines the longest lived isotope, Hg^{203}, because of the relative ease with which it can be detected. This requires irradiation of the samples for 1 week. Using a single channel analyser it is possible to employ the shorter lived pair of activities, Hg^{197m} and Hg^{197}. Then irradiation for three days gives greater sensitivity than the original method. The combination of the reduced period of irradiation and increased sensitivity greatly facilitates the analysis of urine samples, which can be activated and analysed in amounts of the order of 100 mg per sample.

All materials other than urine are dried prior to activation. The digestion of the samples is accomplished in the presence of inactive mercury carrier with a 1:1 mixture of 16N nitric and 36N sulphuric acids. Excess nitric acid is driven off with heat, and the cooled digest transferred with washings to a centrifuge tube for further processing. Mercury is separated from the bulk of interfering activity by almost neutralising the solution with 20% (w/v) sodium hydroxide, and the addition of ascorbic acid. The precipitated mercury is spun down, washed with water and dried with acetone. After all excess acetone is driven off by heat, the metallic mercury is dissolved in 16 N nitric acid. The elimination of all moisture by the

acetone wash ensures that all the metallic mercury is dissolved and present in solution in the mercuric state. Further purification is achieved by the precipitation of silver as the iodide, with the addition of excess iodide. Mercury is precipitated from the supernatant of the silver precipitation by making the solution just alkaline with concentrated ammonia solution, and adding a solution of copper-ethylenediamine. The mercuric iodide/copper-ethylenediamine complex precipitate is washed with water and isopropanol, and transferred to stainless steel planchets for weighing and counting as a slurry in the alcohol.

As stated above counting may be carried out with a single channel analyser set either over the 0.237 MeV photopeak of Hg^{203}, or the 0.077 MeV photopeak of Hg^{197}. The assay of activity can also be achieved using a scaler fitted only with a discriminator to limit the background from the sodium iodide crystal detector. In this case the sensitivity of the analysis is much less than with the slightly more sophisticated pulse analysis system usually employed. All three methods of assaying the mercury activity have been used in obtaining the results presented in succeeding sections.

The cases of exposure to mercury encountered fall into three classes, industrial exposure, acrodynia (Pink's disease) in children from the administration of mercury for medical purposes, and accidental poisoning.

C.2.3. Occupational Exposure to Mercury.

In Table C.8. are shown the results of analyses of hair, nail, and urine from persons working with mercury (A) under normal working

conditions (B) after a period in which the work place is thoroughly cleaned. One subject (B.E.G.) is included, who is not engaged on the same work as the others, and should be free from mercury contamination. It is seen that the degree of contamination varies widely. The general decrease in the mercury content of the samples taken after cleaning the work place indicate that the bulk of the contamination observed initially is mechanical in nature. In the normal values shown for comparison purposes in Table C.9. two sets of means and standard deviations are quoted. Often it is found that the distribution of the values favours the lower end of the series. If the mean and standard deviation are calculated on the basis of the actual values, the asymmetry of the distribution results in a standard deviation which is greater than the mean value. This can be avoided by evaluating the mean and standard deviation in term of the logarithms of the values, giving a geometric mean, which is usually in good agreement with the median value, and a standard deviation which is now a factor by which the mean value should be multiplied or divided to determine the range of values within one standard deviation of the mean value. The use of the logarithms of the values rather than the values themselves implies that the original values lie in a log-normal distribution rather than in a normal distribution.

Dentists and their assistants have been more or less aware of the risks they run in the preparation of amalgam fillings. The results from analyses of samples obtained in a survey of dental assistants are shown in Table C.10. These results have been published by Nixon and Smith (100). In almost all cases the exposed hair and nails show higher contamination than the covered tissue. The general observation is apparent

in the mean values for each type of sample. The mercury content of the covered hair and nail is not significantly different from the normal values for female subjects (Table C.9). No explanation has been found for the difference between the normal mercury content of hair in males and females, although a less marked difference in the opposite sense has been described in the arsenic content of hair by Smith (134). The fact that the difference between mercury content in covered and exposed hair and nail is apparent in the dental assistants and not in the first group of industrial subjects is ascribed to better personal hygiene in the case of the dental assistants. None of the subjects of these investigations shows symptoms of the toxic effects of mercury.

A small sample of hair has been made available, 7 cm in length, purporting to be an authentic specimen of the hair of Charles II of Britain. An analysis carried out on 2 cms of this sample shows a mercury content of 54.6 p.p.m., well outside present day normal values. This is the expected result in view of the well documented assertion that Charles carried out chemical studies in his life time, especially on the fixation, or oxidation, of mercury. As a result his death has been attributed at least partially to exposure to mercury vapour (163).

C.2.4. Acrodynia (Pink's Disease).

A treatment at one time in vogue to assist children in cutting teeth is a mixture of mercury, a sedative, and an inert powder base. The mercury is supposed to assist the teething process

by softening the gums, but often violent side effects are encountered (acrodynia) due to the toxicity of mercury. Consequently the teething powder is not now used. The clinical symptoms of acrodynia are irritability and general malaise, and the excretion of large amounts of mercury in the urine. Treatment of the condition attempts to accelerate the elimination of mercury in the urine, making use of complexing agents such as N-acetyl-D-penicillamine or British anti-Lewisite (B.A.L.). All three cases discussed here have been treated with penicillamine. Urine mercury contents in p.p.m. (μg/ml) from samples before, during, and after, a course of treatment with the complexing agent are set out in Table C.11. Figure C.2. is a graphical representation of the results for one of these cases. Bearing in mind that for children of the age group considered 24 hour urine collections are impracticable, and that the samples are taken as available, some irregularities may be expected in the analytical results due to normal changes in the concentration of the urine. Even so, the results show that during treatment the level of mercury excreted in the urine is higher than before treatment. After administration of the drug is stopped excretion continues much as it did before treatment until normal or near normal urine contents are obtained. The effect of the drug is to accelerate the elimination of mercury, which would occur even without treatment, once the source of the mercury is eliminated. The difference in the children before and after treatment is dramatic. Hair samples from two of the patients taken before the commencement

of treatment give mercury contents of 7.5 and 9.4 p.p.m. and a second sample from one of them taken after the completion of treatment shows a content of 6.0 p.p.m. There is no striking difference between these samples. It appears that the mercury content of hair is not significant in Pink's disease.

Although the children all regained complete health before discharge from hospital, one of them has been reported as having had some recurrence of symptoms. The precise circumstances have not been made clear.

C.2.5. Chronic Mercury Poisoning. (Accidental).

An entire family were treated for mercury poisoning, because the eldest son brought home about 100 g of metallic mercury, which was spilt in the home. The impossibility of adequately cleaning up this amount of metal without expert help is obvious. It is not surprising, therefore, that all members of the household should succumb to mercury intoxication due to air borne contamination some time after the spillage. The entire family were treated with B.A.L. to assist in the excretion of ingested mercury. The diagnosis of mercury poisoning is supported by the mercury content in samples of hair nail and skin taken before the commencement of treatment (Table C.12) in comparison to normal values (Table C.9). After treatment, and all symptoms of exposure to mercury had subsided, the entire family were discharged from hospital. The father and elder son returned to the original home, which had been decontaminated in the meantime, and the mother and the other two children to another house. The father and elder son re-exhibited symptoms soon after

their return. It is not clear whether the recurrence of the symptoms is due to further exposure to mercury, permitted by incomplete de-contamination of the house, or merely due to incomplete treatment with B.A.L. The father and elder son were much the most affected initially, but the remainder of the family who did not re-exhibit symptoms did not return to the contaminated house. The recurrence of symptoms, and the trends observed in the blood and urine mercury content before, and during treatment, and after the recurrence of symptoms, (Tables C.13 and C.14) could equally be explained by the hypothesis that the ingested mercury remains in the body in two distinct forms, rather in the same way that serum copper exists in two forms. There may be a mobile 'pool' of mercury, whose action is the direct cause of the symptomatic appearances, and which is readily removed by the action of the complexing agent used in treatment. The pool may be considered as being in equilibrium with a store of relatively inert mercury, unaffected by the presence of chelating agent. Then treatment eliminates the mobile pool and alleviates the symptoms. If the inert store subsequently replenishes the mobile pool, the symptoms reappear without the patient ingesting any further mercury. This argument can also apply to the Pink's disease cases, where symptoms seem to have re-appeared in the case of one at least of the children. It is extremely unlikely that these children have been exposed to any further mercury.

Examination of the changes in blood mercury content as treatment proceeds, in Table C.13, indicates that there may be two distinct effects in operation. In the case of the father and the two sons

the blood mercury content drops off steadily with time. In the other cases the commencement of treatment has had the effect of increasing the blood mercury content initially. In the samples taken after the recurrence of symptoms it is seen that the father and son, who became ill, show higher mercury content in the blood at this time than the mother who is not affected.

A similar anomaly is observed in the changes in urine mercury content in Table C.14. In some members of the family the urine mercury content rises to a maximum at the commencement of treatment, and then falls off, but in others the values start to fall off as soon as treatment commences. Samples of saliva taken from father, mother and elder son, show very low mercury contents in comparison to the other materials analysed.

C.2.6. The Distribution of Ingested Mercury in Mouse Tissues.

The preceding sections have been concerned with the toxicology of metallic mercury. The following study of the distribution of ingested mercury has been carried out on a mouse given 100 μg of mercury in the form of a solution of mercuric chloride, mixed with whole milk. The dose is not lethal, so that the mouse is sacrificed 24 hours after the ingestion of the mercuric chloride. Analysis of various tissues and organs gives the following results, expressed in p.p.m. dry weight, and arranged in descending order of magnitude:-

Stomach contents - 197.0, Stomach - 79.0, Large Intestine and Contents - 60.1, Kidney - 44.0 Skeletal muscle - 21.0, Spleen - 17.4, Large Intestine without Contents - 17.2, Liver - 4.93, Heart - 1.62,

Lung - 1.45, Blood - 0.89, Bone - 0.715, Brain - 0.519, Urinary Bladder - 0.379, Skin - 0.223, and Teeth - no detectable mercury, representing an upper limit of 0.05 p.p.m.

Aside from the contamination of the digestive tract with unabsorbed mercuric chloride, the compound has been absorbed in the kidney, and in skeletal muscle and to a lesser extent the liver. The blood and the spleen are also considerably contaminated. These results are substantially in agreement with those abstracted from the literature by Bidstrup (12).

C.2.7. Conclusion to Toxicological Applications.

The activation analysis technique supplies a sensitive, accurate, analytical method, applicable on a routine basis, to a wide variety of materials. For toxicological purposes analyses can be achieved by radioactivation which are impossible by classical methods.

C.3. Some Applications of Activation Analysis to Investigation of The Metabolism of Calcium and Strontium.

A problem of interest at the present time is the precise mechanism and kinetics of bone formation in human subjects, especially the part played by calcium and strontium. In a study of this type a powerful tool is the use of labelled material, but the sensitivity of such an investigation is limited by the amount of radioactive material which can safely be administered. The most interesting cases are young children, whose rapid growth and extensive skeletal changes are of especial interest. These subjects should not be exposed to radiation hazards if this can be avoided. Use can be

made at any time of the natural radioactivity in the form of Sr^{90} fall-out, which is steadily appearing in the bone structure of the population. Extensive studies of post-mortem material, relating the amount of Sr^{90} incorporated, to the calcium and stable strontium content, bearing in mind the age of the subject, and the variation in the level of fall-out activity with time, has permitted such workers as Bryant and Loutit (28) to make estimates of the rate of turn over of calcium under various conditions. The material on which their assessment is based has been acquired over a long period of time, and the results of their calculations rest on several assumptions, but the progress made by this kind of study is remarkable.

More direct evidence can be obtained to evaluate the rate at which bone formation takes place by the simple process of estimating the total calcium in-put, the total excretion, and by monitoring the calcium level in the body in terms of the serum calcium level. For this purpose activation analysis has little to offer, in that calcium and strontium can both be measured with greater sensitivity by flame photometry, although there are certain technical difficulties due to interference by other elements. In the course of an investigation of this type the results obtained by flame photometry have been checked by an activation analysis method. Since the strontium and calcium content of the samples involved (ashed diet and faeces) are of the order of a few percent and 100 p.p.m. respectively simple analytical techniques are selected. The estimation

of calcium is attempted by means of the 8.8 minute Ca^{49}. This isotope has a 90% abundant gamma emission at 3.1 MeV, above the energy range of the common activities encountered in the activation of biological materials. Instrumental detection of the calcium activity using a gamma spectrometer is possible. As shown in Table C.15 the analysis is accomplished satisfactorily for samples of ashed faeces, ashed bone, and whole bone, but not in the case of ashed diet, due to interference by large amounts of sodium and chlorine in the samples. It is found to be impossible to eliminate the interference using the usual techniques of spectrum stripping. A technique which has not been applied but which would probably be successful, is the elimination of interference by measuring the sample twice with a time interval between measurements in which Ca^{49} would decay appreciably, but the interfering Na^{24} and Cl^{38} would not. Then the difference between the first spectrum and the second spectrum would be largely due to Ca^{49}. This technique has been used by Liebscher (85) to estimate magnesium and calcium in bone.

A simple strontium analysis is also used, based on the estimation of Sr^{87m}, which has a half-life of 2.8 hours. This isotope emits a 0.39 MeV gamma ray (7%) suitable for gamma spectrometry. At the levels at which strontium is found in the samples supplied, the strontium activity is completely swamped by interfering activity due mainly to Na^{24} and Cl^{38}, so that a chemical separation is required. The half-life of the activity sought is such that a chemical separation is possible. Since a gamma spectrometer is available, providing considerable discrimination against interfering

activities, the chemical separation used is much simpler than that adopted by Bowen (21) where complete radiochemical purity is achieved. After irradiation at a thermal neutron flux of 10^{12} n/ cm^2/sec for 2.8 hrs. the samples are ashed in fuming nitric acid in the presence of strontium carrier. The digest is transferred in 1-2ml of water, and the strontium precipitated by the addition of fuming nitric acid. The precipitated strontium is dissolved in the minimum of water, and precipitated as before by the addition of fuming nitric acid. Finally, the separated material is precipitated as the carbonate, washed, and mounted on aluminium planchets as a slurry in acetone. This simple approach is found to be satisfactory for ashed faeces, ashed bone, whole bone, teeth, but not for ashed diet, due to interference by Mn^{56}. The results are shown in Table C.15. It is a simple matter to carry out both of these analyses on a single sample, provided that the strontium standard is activated along with the samples and the calcium standard in the first analysis for calcium. This is merely to ensure that the strontium standard receives exactly the same irradiation as the samples, without waiting for the activities induced in the samples in the calcium analysis to decay completely before the strontium analysis is carried out.

The agreement between the activation analysis results and the flame photometric results is poor. The activation method is relatively inaccurate because of the peak area computation involved in estimating the activities of calcium and strontium from gamma

spectra. These activation analysis methods can estimate about 1 mg of calcium, and about 1 μg of strontium with an error of 10 to 20 %. The results obtained for the samples of ashed faeces confirm the results obtained with the flame photometer.

Much more elegant experiments can be carried out using tracer techniques. The restricted application of such techniques where radioactive tracers are involved can be overcome by the use of stable tracers. A stable tracer is a stable isotope of a particular element, which has nuclear properties such that it can readily be determined specifically despite the presence of other stable isotopes of the same element and their activation products. It is desirable that the stable tracer isotope should have a low natural occurrence. Calcium and strontium both have stable isotopes suitable for tracer purposes. Ca^{46} is stable, with a natural abundance of 0.0033%, and on activation gives Ca^{47} which is a beta and gamma emmitting isotope with a half-life of 4.7 days, and can be estimated independently of the other calcium isotopes, either by virtue of its half-life which allows time for the interferences to decay, or by gamma spectrometry, which will eliminate interference by Ca^{45}, which is a pure beta emitter. Similarly, Sr^{84} has a natural abundance of 0.56% and the activation product of this stable nuclide (Sr^{85}) can be estimated separately from the other activities induced in the thermal neutron activation of strontium. Although the stable calcium tracer can be obtained in higher degrees of enrichment than the 50% enriched material

available for Sr^{84}, the cost of the calcium tracer is prohibitive. This is partly because more tracer must be given in the case of calcium to produce a significant change in the amount of tracer present in the tissues, in comparison to its normal occurrence. The disadvantage of using the strontium tracer is that the metabolism of calcium and strontium, though broadly similar, differ slightly. For example, the kidney discriminates between calcium and strontium, and this effect must be allowed for in the final evaluation of the results of the tracer experiment.

The techniques of sampling and analysis for the estimation of Sr^{84} in biological material have been described by Smith (139). It is necessary in this case to concentrate the calcium and strontium in the sample before activation. This is easily accomplished by ashing the material, dissolving the ash in dilute nitric acid, and precipitating calcium oxalate in a slightly ammoniacal medium. Under these conditions strontium is co-precipitated quantitatively. If necessary the calcium oxalate precipitate is purified by repeated ashing in nitric acid, and re-precipitation. The pre-separation accomplishes two steps. The weight of oxalate precipitated can be used as a gravimetric estimation of the calcium content of the sample. It also concentrates the strontium in a form suitable for activation.

Activation is carried out for 7 days at a thermal neutron flux of 1.2×10^{12} n/cm^2/sec. The comparison standard is a sample of strontium carbonate, without any enrichment in Sr^{84}, but whose content of this isotope is known. The radiochemical separation

used is a complex series of precipitations. Calcium and barium are separated from the desired strontium activity, by precipitating them as the potassium ammonium ferrocyanide complex, and the chromate respectively. Finally the separated strontium activity, along with the added carrier is precipitated as the carbonate, washed with water and acetone, and mounted on an aluminium planchet using acetone as the suspending medium. Gravimetric recovery estimations for the sample and standard are carried out in the usual way. The Sr^{84} content of the sample is calculated from the activity of sample and standard as measured using a thallium activated sodium iodide crystal detector. Either a single channel or multi-channel pulse analysis system is used to estimate the activity in the 0.513 MeV photopeak of Sr^{85}.

The results of preliminary investigation are shown in Table C.16. A period of four weeks is covered, when all urine and faecal excretion is collected, and a sample of serum taken each week. The patient is given a calcium rich diet over the first two weeks. This is accompanied by a total dose of 500 μg of Sr^{84}. The table shows total faecal and urinary output of calcium (g) and Sr^{84}(μg), and also $\mu gSr^{84}/g$ of calcium, calculated on a weekly basis. The weekly serum values show the calcium and Sr^{84} content in mg% and μg% respectively. The Sr^{84} content of serum is also in mg% and μg% rets of $\mu gSr^{84}/gCa$, as for urine and faeces.

In the case of urine and faeces the concentration of Sr^{84} increases steadily over the first two weeks, and then falls off again. In the case of serum the maximum content of Sr^{84} is not attained until the third week. In all three types of material the ratio of Sr^{84} to calcium attains a maximum value in the third week. This experiment shows that the tracer Sr^{84} is detectable above the level of Sr^{84} normally present, but the data is not available which would be necessary for a more comprehensive study.

In its application to stable tracer experiments of this kind activation analysis is competing in specificity and sensitivity with the mass spectrometer. The sensitivity of the analysis is such that tracer can be used in small quantities, so that there is no fear of the added tracer disturbing the normal metabolic balance.

C.4. Further applications of Activation Analysis to Medical Studies .

C.4.1. Selenium in Retinal Tissue.

It has been suggested recently by Siren (128) that selenium is effective in determining visual acuity. This observation rests on the known photo-electric properties of the element, and the observation that the retinal selenium content varies from one species of animal to another, in parallel with visual acuity. A method is required for the estimation of selenium in a large number of samples. Methods are available,

such as that of Bowen (21) based on the estimation of the pure beta emitting Se^{81}, with a half-life of 18 minutes, which gives the greatest sensitivity for the estimation, but is not really suitable for the analysis of a large number of samples. In any case the chemical separation required is complex because the activity sought is a pure beta emitter. Thermal neutron activation of selenium also produces Se^{75}, with a half-life of 121 days. This isotope decays by electron capture, and emits gamma rays at 0.14, 0.12, 0.27, and 0.28 MeV. Activation of selenium for one week gives a specific activity of 2 mc/g of Se^{75} or about 7 x 10 d.p.s/g Se, or roughly 10^7 counts/s/g Se, adequate for the estimation of 0.1 g of selenium. The use of the long lived activity greatly simplifies the problem of radiochemical separation, because short lived contaminants can be allowed to decay, and others eliminated by gamma spectrometry. In place of the complex precipitation and distillation separation of Bowen, adequate radiochemical purity is obtained by digesting the samples in 1:1 16N nitric acid, 36N sulphuric acid mixture, and precipitating the selenium from the digest by reduction with ascorbic acid. After allowing 10 days to elapse to complete the decay of Na^{24} and other short lived interferences, counting with a single channel analyser set over the 0.12 and 0.14 MeV photopeaks of Se^{75} gives satisfactory results. It is found to be necessary to transfer the precipitated selenium to planchets using ethanol, because the precipitate reacts vigorously with acetone.

The results of the analyses cannot be interpreted as the identity of the samples has not been revealed. Solid samples show selenium contents of from 2.0 to 60.0 p.p.m. (dry weight). Solutions show contents of zero to 3.0 μg/ml. One result of interest is the detection of 3.0 p.p.m. selenium in the liquid paraffin employed in the preparation of some of the samples.

This study shows how a very simple activation analysis method can be devised to meet the requirement of a specific problem.

C.4.2. Clinical Analyses for Haemochromatosis.

This problem requires the estimation of copper, zinc, and manganese, in liver and blood from a suspected case of haemochromatosis. Control samples from normal subjects have also been analysed. Copper and zinc are determined in single samples using the isotopes Zn^{69m} and Cu^{64}, and a chemical separation combining the methods for copper and zinc described by Bowen (21). Manganese is determined using the isotope Mn^{56}. In this case a simple separation involving digestion of the sample with 16N nitric acid, followed by the precipitation of manganese dioxide by the addition of sodium chlorate serves to produce the manganese activity in a suitably pure form for its estimation by gamma spectrometry. At the levels found in human tissues, a single channel analyser can be used to estimate the manganese activity to within 10%, although detectable amounts of Na^{24} contaminate the separated material. Decay curve analysis indicates that the degree of contamination is well under 10% in a typical sample, after activation for 30

minutes.

The results of the activation analysis methods are shown in Table C.17. along with results of a similiar investigation from the literature. The activation analysis results indicate that haemochromatosis produces deficiency in the blood of both copper and manganese, with a less marked deficiency in the liver. The zinc content of the blood seems to be elevated at the expense of the liver. These generalisations summarise the results in Table C.17, but the available data are rather few for drawing significant conclusions.

C.5. Applications of Activation Analysis to Forensic Science.

C.5.1. Detection of Gunshot Residues.

It is known that in firing a gun, whether a pistol, a rifle, or even a shot gun, the person who fires the gun is contaminated by residues from the primer and propellant. In the original form of the test the residues are lifted from the suspect's hand in the region of the webb of the hand, between thumb and forefinger using a paraffin wax film, and the wax surface in contact with the hand tested for the presence of oxidising agents, using diphenylamine. The test with diphenylamine is not specific, nor is it reliable. The application of activation analysis to this test is described by Guinn et al (116), where the use of diphenylamine is replaced by activation analysis for antimony and barium, two common components of primers and propellants. Subsequently the technique has been expanded to include the estimation of copper (117). Scott et al (124) have published an account of their evaluation by high speed photography of the method whereby the person who fires the gun is contaminated. They also describe their method

for determining lead, antimony, and barium, on the hands of subjects after they have fired various types of weapon.

In this study the examination is confined to antimony and barium. The powder residues are removed from the webb of the hand of the suspect by forming a cast of paraffin wax by brushing on the molten wax (melting point about 40°C) in thin even layers, using a clean camel hair brush. After the wax has hardened the cast is carefully removed care being taken to avoid its contamination by handling. The material lifted from the skin is prepared for irradiation by scraping off about 0.5 mm of the surface of the cast which has been next to the skin of the suspect, using a fresh stainless steel scalpel blade for each sample, to avoid cross contamination. The scrapings are packed in polythene vials for irradiation. Also included are a blank sample obtained by scraping the outside of one of the casts, and standard samples of antimony (0.1 μg) and barium (1.0μg), prepared by evaporating small volumes of standard solutions of these elements onto clean polythene sheets. To avoid contamination during irradiation, each sample and standard is wrapped in a small polythene envelope, which is heat sealed. The samples and standards are activated together for 1 hour at a thermal neutron flux of 10^{12}n/cm^2/sec. Immediately after the end of the irradiation the samples and standards are unpacked, and the activated material transferred to a conical flask, containing 25 ml 2 N hydrochloric acid, and in the case of the samples 20 mg each of barium and antimony carrier are added. In the case of the standards, only the appropriate carrier is present. Heating with constant stirring permits the extraction

of the antimony and barium activities from the wax, along with Na^{24} and Cl^{38} activity from the activation of the normal sodium and chlorine skin contamination. Sodium hold-back carrier is added in the form of sodium chloride. After transferring the aqueous extracts to centrifuge tubes, barium is precipitated by the addition of sulphuric acid. The supernatant is carefully poured off, and retained for the isolation of antimony, as described in the antimony method (section B.2). The barium precipitate is washed with water and acetone in the usual way, and transferred with acetone to an aluminium planchet for weighing to determine the chemical recovery. The gamma spectrometric estimation of the induced Ba^{139} activity is achieved using the 0.16 MeV photopeak. No other major activity appears in the gamma spectrum of the samples, but much extraneous beta activity is found if a Geiger counter is used. The activity isolated in the antimony fraction can be estimated by Geiger counting if the full separation described in section B.2 is used. The use of gamma spectrometry permits the estimation of the antimony activity without a full scale separation.

The separation described above is applied to casts from the hand of a volunteer (E.R.) who fired a service revolver twice. One wax cast is taken after the first single shot, and the other after the firing of two successive shots. At the same time casts are made from the palm and fingers of H.S. who handled the gun on each occasion after it had been fired. No detectable barium or antimony activity is found in the blank wax sample prepared from the reverse side of one of the casts. The levels of contamination found on the

sides of the casts next to the skin of the subjects are shown in Table C.18. It is seen that the degree of contamination is much the same in both the subject who fired the weapon, and the subject who merely handled it, although, as expected the area which is contaminated is different in the two subjects. The amount of barium and antimony found in both subjects is greater after two shots have been fired than after the firing of the single shot. The analysis shows a rough quantitative relationship between the degree of contamination by powder residues and the number of shots fired. The contamination of the subject who only handled the gun after firing makes the point that the weapon itself is contaminated in much the same way, and this contamination can be transferred to the skin on contact. A further complication is the variation in the composition of propellant and primer and hence the variation in the elemental content of the powder residues in different types and makes of ammunition. The elements involved in the estimation are not uncommon, so that it may be expected that people who have not fired a weapon will be contaminated with them in any case. Guinn (116) has assessed the normal skin contamination of these elements at $0.13 \pm 0.11 \mu g$ Ba and $0.015 \pm 0.012 \mu g$ Sb. These normal values are of the same order of magnitude as the values found in contaminated subjects in this work, but the experimental values do show the expected trend. Lastly, it has been pointed out that the contamination from firearm discharge is not permanent. Hence a claim that a wrongful accusation had been made because the suspect fired a weapon on a previous occasion to that for which he is indicted is not

admissable.

A similiar micro-analysis, applied only to the estimation of antimony has been suggested by Baumgartner et al (9) to extend the range of distances over which the distance between a victim and a weapon can be estimated beyond that determined by the area of powder burn on the victim.

C.5.2. Multi-element Instrumental Analysis and Hair identity.

An important application of activation analysis in the field of forensic science is the estimation of trace elements with a view to establishing the identity of a sample of material. The elements involved are present in the material in the form of accidentally included contamination, and reflect the history of the material. Materials to which this type of investigation is applicable include glass, paint, oil, grease, narcotic drugs, earth and hair. The application to hair is of especial interest because it is suspected that the successful application to this material may provide a means of absolute identification at least as specific as finger printing.

The approach to the problem is to activate hair samples for various times and determine the gamma spectrum of the activated sample at various intervals after each irradiation. Other laboratories (106) are expending a great deal of effort in massive surveys of hair from large populations to evaluate the technique with regard to the number of elements detectable, and the levels at which they occur.

In a preliminary study two separate activation periods are used, 30 minutes and 24 hours. In the shorter irradiation period

the spectra showed peaks attributed to Na^{24}, Cl^{38}, Cu^{64} (0.51 MeV, positron annihilation), and in some cases Mn^{56} (Figure C.3). After the longer irradiation, Na^{24} was observed in all samples, but peaks attributed to Au^{198}, and Hg^{197}, are observed in certain samples. The samples investigated include a sample from the scene of a crime, samples of hair from two suspects, and several control samples. In fact the evaluation of the gamma spectra gives inconclusive results. No pair of samples give identical spectra under the same conditions of irradiation and measurement, nor are the differences observed great enough to refute possible identity. The problem in a study of this kind is the presentation and evaluation of the mass of data obtained from the spectrometer. In this case the observations are based on the visual comparison of the spectra obtained, and an attempt to reduce the volume of data to a numerical evaluation of the spectra. The numerical approach is based on the estimation of the areas of the various peaks related to the area of a particular reference peak selected arbitrarily. The results of this type of computation for the preliminary experiment are shown in Table C.19. It is not necessary for proof of identity to know what elements the peaks represent, nor even the gamma energies producing them, although their position in the gamma spectrum is important.

In this experiment the stumbling block is the activity due to Na^{24} and Cl^{38}, which is the bulk of the observed activity. The amount of these activities follows no logical relationship to the samples. It may be that the amount of sodium and chlorine in the hair are parameters which should be neglected for identification

purposes on the grounds that the presence or absence of dried sweat affects the apparent hair content significantly. Two possible approaches may be considered. One is to eliminate the Na^{24} and Cl^{38} activities from the spectra and base the comparison for identification purposes on the remaining activities. This is not a simple task, because of the high proportion of the total activity which is due to these unwanted elements. Again, some cleaning process may be applied to the samples before activation to eliminate trace elements which are not an integral part of the structure of the hair . The problem here is the selection of a suitable agent which will remove the extraneous contamination without affecting the significant material of the hair, and without depositing further contamination. Most workers employ some form of pre-irradiation cleaning in hair analyses, using water, detergent solution, organic solvents, or a combination of these. Before any extensive study of this problem can be carried out major difficulties in the collection and evaluation of data must be overcome. In the use of the gamma spectrometer it is imperative that the resolution and energy calibration of the instrument does not change over the period of time during which a series of spectra are measured. Careful control of the conditions under which the spectrometer is used can help to minimise this effect. In evaluating the data from the spectrometer some form of data reduction is imperative. The most promising approach is to carry out a mathematical analysis of the spectra using a computer. The mathematical approach can also be employed in correcting the spectra for gain shift, observed as a change in the position and half-width of a peak common to all the

spectra such as that of Na^{24}. Several workers have in operation activation analysis systems involving computer analysis of gamma spectra. The equipment involved is complex, and the design of the mathematical approach to produce the maximum information from the data is difficult.

C.6. Normal Trace Element Content of Human Tissues.

Investigation of the function of trace elements in health and disease states requires a knowledge of the normal trace element content of the biological system. A programme of analysis of normal tissues derived from post-mortem examinations is being carried out. The deaths have occurred as a result of traffic accidents, falls, and other similiar occurrences, and are not due, as far as is known, to disease. The subjects from whom the samples are taken all livedand worked in and around the city of Glasgow. The aim of the programme is to achieve the analysis of samples of all the major organs from twenty separate cases for each element investigated. Results are presented representing the progress so far in the estimation of normal tissue levels for antimony, arsenic, copper, and mercury.

Aside from the actual analytical process, one of the main difficulties of an investigation of this type is the interpretation and presentation of the results, and especially an accurate estimation of the true normal range for a particular element in a particular organ. The situation is complicated by the wide ranges of contents which are observed. This is a legitimate observation, and not due to analytical errors. The effect may be due partly to the variation in the concentration of a particular element in a specified organ of a single

individual. Even if care is taken to analyse anatomically and physiologically identical portions Molokhia (96) has observed differences of an order of magnitude in the arsenic or antimony content of single samples of lung or liver analysed in replicate. Still greater differences are observed between the trace element contents of portions of the same anatomical organ with different physiological function. The simple answer to this problem is the analysis of a large sample to give an average value for the organ. This requires the digestion of large amounts of organic material, which is not convenient. As a compromise the analyst can attempt to select the portions of material analysed, in order to obtain representative values. The advantage of being able to obtain more representative samples from homogenized material is offset by the hazard of contamination during the grinding, or mincing, and mixing process. The same risk of contamination, together with the possibility of losing volatile material, including trace elements, casts doubt on the application of dry ashing as a means of concentrating the mineral content of the samples, and producing homogeneous material for analysis.

In determining the ranges for normal values, the information of the maximum and minimum value obtained is not sufficient, because the distribution of the results between these values is significant. The same argument applies to arbitrarily determined confidence levels where the 95% confidence range is that region in which 95% of the experimental values lie. The only satisfactory way to describe the range of results is to quote a reference value and a probable error of some kind. If the results lie in a normal distribution, symmetrically

distributed about a mean value, then it is easy to describe the range of the results in terms of the mean and the standard deviation for that distribution. If, as is the case in most of the results presented here, there is a bias to low results, then the evaluation of the arithmetic mean and the associated standard deviation often produces a mean smaller than the standard deviation, which is meaningless. At the same time it is found that the mean value is quite different from the median value. In this situation the application of the mean and standard deviation calculation to the logarithms of the values, thus assuming that the distribution of the results is log-normal, results in a mean value much closer to the observed median value. If the calculation of the standard deviation is carried out on the logs of the values, on taking anti-logs of the mean and standard deviation to return to the same scale as the experimental results, the standard deviation becomes a factor by which the mean value must be multiplied to determine the range. For example, in a normal distribution with an arithmetic mean M, and a standard deviation D, 95.5% of the values will lie in the range (M-2D) to (M+2D). In a log-normal distribution, on the other hand, where the antilogarithm of the mean of the logarithms of the experimental values is M_1, and the antilogarithm of the standard deviation of the logarithms of the values is D_1, then 95.5% of the values will lie in the range $M_1/(D_1)^2$ to $M_1.(D_1)^2$.

In deciding whether to accept a set of results as normal or log-normal the following criteria can be considered, all based on a decision as to symmetry of the distribution of the values around the mean. First if the distribution is symmetric then the mean, and

median values coincide. Again, in a symmetric distribution, the quantity ϱ, defined as the sum of the cubes of the deviations of the individual results from the mean value, divided by the number of results times the cube of the standard deviation, will be zero. A decision as to whether a set of results fit better to a normal distribution or to a log-normal distribution, may be made on comparison of the agreement of the mean and median values as computed from the original values and their logarithms. The same test can be applied graphically, by plotting the values on a linear scale, and again on a logarithmic scale. The asymmetry observed in the linear plot, and the symmetry observed in the logarithmic plot suggests that the results lie on a log normal distribution. This effect is shown for the arsenic content of adrenal in Figure C.4.

In the results shown in Table C.20. the results fit better to the log-normal distribution for all the tissues analysed for arsenic, antimony, and mercury. All the sets of figures for copper on the other hand, except for hair and nail, fit normal distributions. The limited data obtained so far does not permit a decision to be made as to whether the exception to the general distribution rules are legitimate observations, or artefacts arising from the limited nature of the data. Hair and nail are subject to extensive contamination from cosmetics and other sources. Wester (166) has observed that trace elements with a metabolic function in a tissue tend to have a narrower range of concentrations in a series of analyses, than elements which have no function. On the basis of the results presented here it appears that an alternative statement would be

that the analysis of an essential trace element in a series of samples of a given organ will produce a normal distribution of results. A non-essential element will give a log-normal distribution. Hence the form of the distribution of the results is an indication of the essential or non-essential nature of the element. Here essential merely means that the element has a specific place in the biochemistry of the organ. This is not the same as the more usual definition of an essential trace element, which is defined as being essential to life. This criterion of distribution of results may act as an indicator for those elements for which further investigation is worthwhile.

The values obtained by activation analysis are in broad agreement with those of other workers using other analytical techniques, bearing in mind that the activation analysis results in Table C.20 are expressed as p.p.m. (dry weight). Other estimations of the normal copper content of tissues are found in the work of Butt et al (57) and Tipton et al (107). The results for arsenic and antimony are in agreement with the limited data available in the "Committee 2 report" (113). Previous work on the mercury content of normal tissue is sparse, but some of the recent work is summarised in Table C.21.

A very large discrepancy is observed between the normal trace element content of hair as determined by the single element analyses, and the values reported by Perkons and Jervis (106). The latter results are about an order of magnitude higher than the values obtained by the single element analysis, for all the elements presented in Table C.20, with the exception of mercury. The differences seem rather larger than can be explained as due to the different areas in

which the subjects, who provided the samples for analysis, lived.

C.7. Comparison of Trace element content of Normal and Cancerous Tissue

In parallel to the study of normal tissues the same four elements with the addition of manganese have been determined in cases of lung cancer. Separate analyses were carried out for each element in the carcinoma, the involved lung, and the uninvolved lung. The median values (p.p.m. dry weight) are listed in Table C.22. In all elements the carcinoma value is very low compared to the other two tissues. However the involved lung is richer in all the trace elements than the uninvolved lung with the exception of antimony. This is very similiar to the findings of Tietz et al (153). The low trace element content of the carcinoma is entirely expected, since this is tissue which is developing rapidly.

C.8. Some Further Investigations on Arsenic in Biological Materials

C.8. Arsenic and Cancer

The carcinogenic properties of arsenic have been established for some time, and cases of lung cancer observed to occur to an unusual degree among persons who are occupationally exposed to arsenic. One such group are tin smelters and analysis of hair and nail samples from this group give very high contents of arsenic. The results of the analyses for hair lie in the range 0.08 to 753.0 p.p.m. with a median value of 32.0 p.p.m. Nail values range from 0.0003 to 829.0 p.p.m. with a median value of 29.1 p.p.m. It is reasonable to suspect that the high incidence of lung cancer in this group of the population is related to the large amounts of arsenic to which they are exposed,

assuming that their lungs are contaminated in a similiar fashion to their hair and nails. Analysis of a sample of lung from a retired tin smelter, who died of lung cancer shows an arsenic content of 1.6 p.p.m. (dry weight), about twenty times the normal value. This tends to support the hypothesis given above. Analysis of hair, nail, kidney, and liver samples from a second deceased retired tin smelter, where the values are 0.845, 0.404, 0.037, and 0.034 p.p.m. (dry weight) respectively, show that the contamination with arsenic is superficial, and that the people exposed absorb very little if any arsenic into their bodies.

A further investigation of the relationship between arsenic and lung cancer involves the estimation of the arsenic content of the hair of lung cancer cases diagnosed by mass radiology. To estimate the level of arsenic contamination to which each of these subjects is exposed, a dust sample from their homes or clothing is analysed. Although the relationship between the hair and dust values for each case has not been evaluated statistically, the hair values lie in the normal range described by Smith (135), with a range of 2.19 to 0.02 p.p.m. and a median value of 0.35 p.p.m. The values obtained for the dust samples lie in the range 618.0 to 0.22 p.p.m. with a median value of 5.10 p.p.m. The tin smelters' hair and nail, and the hair and dust samples from the radiological survey, all fit log-normal distributions.

In investigating the possible role of arsenic in the relationship between smoking and lung cancer, a survey of the arsenic content of cigarettes in the late 1950's showed levels around 10 p.p.m. This

was partially due to the use of arsenical pesticides. Due to the discontinued application of arsenicals, a similiar survey carried out in 1964-65, by activation analysis, on samples of tobacco products including cigarettes, cigars and pipe tobacco, shows no value greater than 1.6 p.p.m. The majority of the results are in the range 0.5 to 0.01 p.p.m.

The results in this section are a continuation of work initiated by Lenihan and Smith, and outlined by Lenihan (83). Lenihan et al (84) have described the presence of high concentrations of arsenic in certain detergents, due to impure raw materials used in their manufacture. A repeated survey of British detergents carried out in 1964-65 shows that none of the materials examined contained more than 4.0 p.p.m. and the majority of the samples contained less than 1.0 p.p.m. of arsenic.

C.8.2. Acute Arsenic Poisoning in Cattle.

The death of some cattle was suspected of being due to acute arsenic poisoning. Samples of liver and kidney showed arsenic contents of 5.0 and 2.0 p.p.m. respectively. Some months later hair from the tail of a beast which survived the ingestion of arsenic was obtained and analysed in sections each 1 cm long. The results (Fig. C.5.) show a maximum arsenic content in the seventh cm section from the proximal end of the hair. This observation fits quite well with the time interval between exposure and the time at which the sample was taken, assuming a growth rate for the hair of 1.3 cm per month, as described by Smith (135) as being applicable to human hair. Two regions of

relatively high arsenic content are present in the hair nearer the distal end than the maximum value. These are due to the presence of excreta which is rich in arsenic.

D. Conclusion

In the preceding section, describing applications of activation analysis, it is clear that the technique has wide applicability in many fields where trace element analysis is of value. In medicine increasing interest is being shown in trace elements and their role in disease states. The sensitivity of activation analysis techniques makes possible more sophisticated investigations of the distribution of trace elements at the sub-cellular level, although undoubtedly problems will be encountered in preparing suitable samples for this type of investigation. The most sensitive form of the method, using complex chemical separations, and relatively simple activity estimation techniques can be adopted readily by analysts engaged in work in any of a number of fields. Using samples of a reasonable size complex laboratory facilities are unnecessary, provided simple precautions are taken against the hazards of spillage. Only if complex gamma spectrometry is to be carried out, requiring the use of expensive equipment, is there any reason why thermal neutron activation analysis should not be widely adopted.

The source of the methods applied in radiochemical separations is the general bulk of analytical chemistry. The modifications required for their use in activation analysis are generally slight, and the advantages of separations using carrier amounts of material, are strongly in favour of the activation analysis method. In addition, the freedom from the hazards of contamination during chemical processing, and the demonstrable specificity of any given

analysis, both commend the application of the activation technique.

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TABLE A.1.

Comparison of Sensitivities for the Detection of the Elements by Thermal Neutron Activation Analysis, Flame Photometry, Colorimetry and Amperometric Titration.

Sensitivities are expressed as the minimum concentration of each element in solution (microgram per ml) which are detectable, assuming that 25 to 50 ml of solution is available.

The figures for activation analysis assume that 1 ml samples are irradiated to the saturation activity level or for 30 days, which ever is the shorter, the flux is 5×10^{11} thermal neutrons/cm^2/sec, and the minimum detectable activity requires 40 disintegrations per minute. No account is taken of chemical separation procedures or interferences in any method. For the activation technique this means that the decay of the induced activity while such separations are accomplished is neglected.

Element	Activation Analysis	Flame Photometry	Colorimetry	Amperometric Titration
H				
He				
Li		0.02		
Be		250.0	0.04	
B		10.0		
C				
N				
O				
F				0.25
Ne				
Na	0.007	0.002		
Mg	0.6	1.0	0.06	
Al	0.001	20.0	0.002	300.0
Si	1.0		0.1	
P	0.02		0.01	15.0
S	4.0			5.0
Cl	0.03		0.04	10.0
A				
K	0.08	0.01		100.0
Ca	3.8	0.03		100.0
Sc	0.002			
Ti		2.0	0.03	10.0
V	0.001	2.0	0.2	3.0
Cr	0.2	1.0	0.02	1.0
Mn	0.0006	0.1	0.001	0.0003
Fe	9.0	2.0	0.05	2.0
Co	0.02	10.0	0.025	100.0
Ni	0.03	10.0	0.04	0.5
Cu	0.007	0.1	0.03	10.0
Zn	0.04	2000.0	0.016	10.0
Ga	0.007	1.0		
Ge	0.04		0.08	

TABLE A.1(continued):-

Element	Activation Analysis	Flame Photometry	Colorimetry	Amperometric Titration
As	0.0002		0.1	0.4
Se	0.05			
Br	0.003			200.0
Kr				
Rb	0.03	0.1		
Sr	0.6	0.1		
Y	0.01	50.0		
Zr	0.3		0.13	
Nb	10.0	20.0	50.0	
Mo	0.1	30.0	0.1	5.0
Tc				
Ru	0.1	10.0	0.2	
Rh		1.0	0.2	
Pd	0.005	1.0	0.1	
Ag	0.11	0.5	0.1	1.0
Cd	0.05	20.0	0.01	5.0
In	0.0001	1.0	0.2	100.0
Sn	0.2	10.0		2.0
Sb	0.004		0.03	10.0
Te	0.1	100.0	0.5	
I	0.002			1.0
Xe				
Cs	0.03	1.0		
Ba	0.05	3.0		25.0
La	0.002	5.0		
Ce	0.1	20.0	0.25	500.0
Pr	0.002	100.0		
Nd	0.1	50.0		
Pm				
Sm	0.0006	100.0		
Eu	0.00003			
Gd	0.02	10.0		
Tb	0.004			
Dy	0.00003	10.0		
Ho	0.0004			
Er	0.02			
Tm	0.002			
Yb	0.002			
Lu	0.0003			
Hf	0.02			
Ta	0.007			
W	0.003		0.4	
Re	0.0006		0.05	
Os	0.02		1.0	
Ir	0.0003		2.0	
Pt	0.1		0.2	
Au	0.003	200.0	0.1	
Hg	0.13	100.0	0.08	
Tl	0.6	1.0		

TABLE A.1 (continued):-

Element	Activation Analysis	Flame Photometry	Colorimetry	Amperometric Titration
Pb	2.0	20.0	0.03	3.0
Bi	0.4	300.0	1.0	300.0
Po				
At				
Em				
Fr				
Ra				
Ac				
Th				
Pa				
U	0.01	10.0	0.7	

TABLE A.2.

Elements Classified According to the Sensitivity of their Detection by Activation Analysis According to Table 1.

Sensitivity	Elements
$\leq 10^{-4}$ µg/ml	Dy, Eu, In.
$\leq 10^{-3}$ to 10^{-4} µg/ml	Al, Ho, Ir, Lu, Mn, Re, Sm, V.
$\leq 10^{-2}$ to 10^{-3} µg/ml	As, Au, Br, Cu, Ga, I, La, Na, Pd, Pr, Sb, Sc, Ta, Tb, Tm, W, Y, Yb.
$\leq 10^{-1}$ to 10^{-2} µg/ml	Ba, Cd, Ce, Cl, Co, Cs, Er, Gd, Ge, Hf, K, Mo, Nd, Ni, Os, P, Pt, Rb, Ru, Se, U, Zn.
≤ 1.0 to 10^{-1} µg/ml	Ag, Bi, Cr, Hg, Mg, Si, Sn, Sr, Te, Tl, Zr.
≤ 10 to 1.0 µg/ml	Ca, Fe, Nb, Pb, S.
No sensitivity quoted	Ac, Ar, At, B, Be, C, Em, F, Fr, H, He, Kr, Li, N, Ne, O, Pa, Pm, Po, Ra, Rh, Tc, Th, Ti, Xe.

Compare the above with the following table from Gibbons (51), where the thermal neutron flux is assumed to be 10^{12} n/cm^2/Sec.

Sensitivity	Elements
$< 10^{-4}$ µg	As, Au, Br, Cu, Dy, Er, Eu, Ga, Gd, Ho, In, Ir, Kr, La, Li, Lu, Mn, Na, P, Pd, Pr, Re, Sc, Sm, Ta, Tb, Tm, W, Yb.
10^{-2} to 10^{-4} µg	A, Ba, Cd, Ce, Cl, Co, Cs, Ge, Hf, Hg, I, K, Mo, Nd, Ni, Os, Pt, Rb, Ru, Sn, Te, Th, Tl, U, Xe, Y, Zn.
10^{-1} to 10^{-2} µg	Bi, Ca, Cr, Fe, O, S, Se, Si, Sr, Zr.

TABLE A.3.

Elements for which Classical Chemical Analysis is more Sensitive than Thermal Neutron Activation Analysis (based on Table A.1.) Sensitivities in μg/ml.

Element	Activation Analysis	Flame Photometry	Colorimetry
Na	0.007	0.002	
P	0.02		0.1
Zn	0.04		0.016
Cd	0.05		0.01
K	0.08	0.01	
Ag	0.11		0.1
Hg	0.13		0.08
Cr	0.2		0.02
Zr	0.3		0.13
Mg	0.6	1.0	600.0
Sr	0.6	0.1	
Si	1.0		0.1
Pb	2.0		0.03
Ca	3.8	0.03	
Fe	9.0	2.0	0.03

TABLE B.1.

Comparison of Techniques Available in Radiochemical Separations after Bowen and Gibbons (25) and Thomson (152).

Technique	Time Required (min)	Selectivity	Concentration Dependance	Applicability
Volatilisation	1	Good - Excellent	None	Non-metals
Solvent Extraction	1	Moderate - Good	None	Most Elements
Chromatography	30	Good - Excellent	None	Most Elements
Precipitation	2	Poor - Good	Strong	Most Elements
Electrodeposition	30	Excellent	None	Some Elements
Mass Spectrometry	10	Excellent	None	All Elements

Neutron Capture Properties of Antimony, and Possible Interfering Reactions from Allen et al (2).

Target Isotope	Cross-section (barns)	Product Isotope	Half-life of product	Principal Emissions (MeV) (Abundances given in parenthesis)	Specific (e) Activity per g target
Sb^{121}	3.9 (a)	Sb^{122}	2.74 d	β 0.73, 1.42 (63%), 1.99 (30%) γ 0.57 (66%)	290 mc/g
Sb^{123}	1.07 (a)	Sb^{124}	60.0 d	β 0.22 (11%), 0.61 (51%), 2.31 (23%). γ 0.60 (99%), 1.70 (50%).	5.0 mc/g
I^{127}	0.018 (b)	Sb^{124}	As above	As Above	8.0 μc/g
Sn^{124}	0.012 (c)	Sb^{125}	2.0 y	β 0.12 (29%), 0.30 (45%), 0.44 (12%), 0.61 (14%). γ 0.427, 0.595	~~3~~ 4.5 μc/g (d)

(a) Thermal neutron capture cross-section of the natural element.

(b) Cross-section for the (n, α) reaction for 14.3 MeV neutrons taken from Hughes and Schwartz (69).

(c) Cross-section of the natural target element for Sb^{125} production using pile neutrons. The reaction initially produces Sn^{125}, which decays with the emission of beta particles, with a 10 min. half-life to give the final product.

(d) Figure calculated assuming fast neutron flux of the reactor is 1/10th of the thermal neutron flux.

The short lived metastable antimony isotopes have been neglected in this discussion.

(e) Specific activities calculated on the basis of 3 days irradiation at a thermal neutron flux of 1.2×10^{12} n/cm^2/sec.

TABLE B.3.

Conditions Governing the Precipitation of Antimony by Chromous Chloride.

HCl (molarity)	H_2SO_4 (molarity)	16 M HNO_3 (drops)	Heating Time (minutes)	$CrCl_2$ (ml) (Approx. 1M.)	Sb Precipitated (%)
0.5	3.6		2	2.0	93.0
1.1	3.6		2	2.0	91.0
1.6	3.6		2	2.0	90.0
2.7	3.6		2	2.0	90.0
0.5	0.0		2	2.0	91.0
0.5	3.6		2	2.0	93.0
0.5	7.2		2	2.0	94.0
0.5	14.4		2	2.0	93.0
0.5	3.6	1	2	2.0	72.0
0.5	3.6	2	2	2.0	70.0
0.5	3.6	4	2	2.0	70.0
0.5	3.6	8	2	2.0	69.0
0.5	3.6		1	2.0	93.0
0.5	3.6		2	2.0	93.0
0.5	3.6		5	2.0	92.0
0.5	3.6		2	0.5	10.0
0.5	3.6		2	0.75	30.0
0.5	3.6		2	1.0	45.0
0.5	3.6		2	1.5	63.0
0.5	3.6		2	2.0	93.0
0.5	3.6		2	3.0	98.0

TABLE B.4.

Conditions Governing the Precipitation of Arsenic by Hypophosphite.

HCl (molarity)	Heating Time (minutes)	Ammonium Hypophosphite (Saturated solution at 20°C) (ml)	As Precipitated (%)
2.2	5	2.0	17.4
3.3	5	2.0	29.0
4.4	5	2.0	54.5
6.6	5	2.0	59.7
6.6	5	2.0	59.7
6.6	10	2.0	75.5
6.6	15	2.0	87.6
6.6	20	2.0	99.0
6.6	20	0.25	77.8
6.6	20	0.5	91.5
6.6	20	1.0	97.1
6.6	20	2.0	99.0

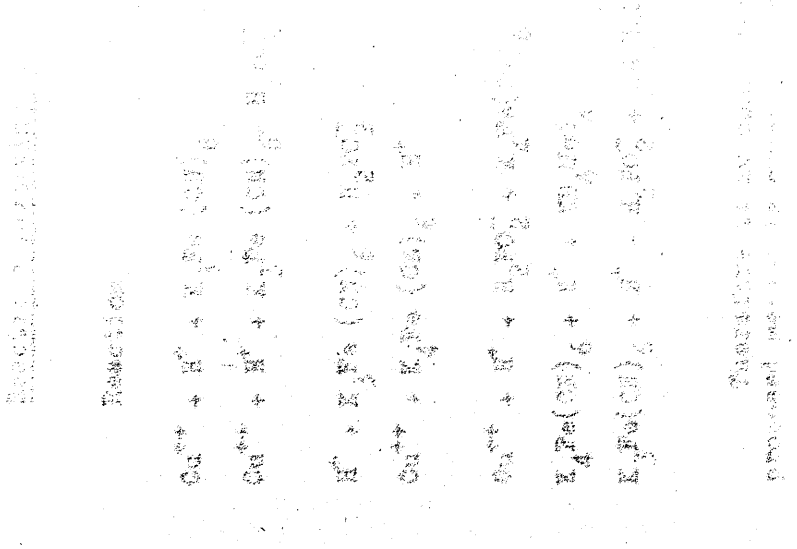


TABLE B.5.

Reactions involving Copper, Hypophosphite, and Ferricyanide.

Reaction	Precipitate Colour	Comment
$Cu^{++} + H^{+} + K_3Fe(CN)_6$	Yellow-green	
$Cu^{++} + H^{+} + K_3Fe(CN)_6 + H_2PO_2^-$	Brown	Conditions for copper precipitation in Sb method.
$H^{+} + K_3Fe(CN)_6 + H_2PO_2^-$	No Precipitate	
$Cu^{++} + K_4Fe(CN)_6 + H^{+}$	Brown	Possibly the same as in the proposed method.
$Cu^{++} + H^{+} + H_2PO_2^- + K_4Fe(CN)_6$	Pink	
$K_4Fe(CN)_6 + H^{+} + NH_4MoO_4$	Red	Test for ferrocyanide.
$K_3Fe(CN)_6 + H^{+} + H_2PO_2^- + NH_4MoO_4$	No Precipitate	Hence ferricyanide is not reduced by hypophosphite in acid solution.

Therefore it is concluded that the precipitate in which copper is eliminated in the proposed method is cuprous ferricyanide.

TABLE B.6.

Conditions governing the Precipitation of Copper as Cuprous Ferricyanide.

Temperature of the solution (°C)	Hypophosphite solution saturated at 20°C. (ml)	HCl (molarity)	$K_3Fe(CN)_6$ 10% w/v (ml)	%age Cu retained in solution.
60	1	0.5	0.25	2.2
40	1	0.5	0.25	1.3
20	1	0.5	0.25	0.4
20	1	0.5	0.25	0.4
20	2	0.5	0.25	1.2
20	5	0.5	0.25	0.8
20	1	0.5	0.25	0.4
20	1	1.1	0.25	7.2
20	1	2.7	0.25	8.6
20	1	0.5	0.1	30.4
20	1	0.5	0.25	0.4
20	1	0.5	0.5	0.0

TABLE B.7.

Ratio of Gamma counts to Beta Counts Showing Contamination by P^{32}.

Sample No.	Beta count (c/m)	Gamma count (c/m)	Ratio Beta count/gamma count
1	928.0	78.8	11.8
2	4250.0	1021.0	4.15
3	1953.0	450.0	4.34
4	478.0	66.6	7.25
5	1121.0	54.9	24.0
6	1403.0	97.6	14.4
7	2876.0	404.0	7.13
8	2810.0	62.9	44.7
Standard	4260.0	1003.0	4.15

Both beta and gamma activities are corrected for counter dead time and chemical recovery.

TABLE B.8.

Comparison of Results by Beta and Gamma Detection after either Careful Washing or Phosphate Precipitation.

	Sample	p.p.m. Beta	p.p.m. Gamma
Careful Washing	Tooth	0.023	0.023
	Lung	2.26	2.27
	Lung	1.69	1.67
	Liver	0.063	0. 065
Phosphate Precipitation	Tooth	0.018	0.020
	Lung	1.59	1.56
	Lung	1.80	1.75
	Liver	0.037	0.041

Values expressed as p.p.m. dry weight.

TABLE B.9.

Trace Element Content of Dried Kale according to H.J.M. Bowen (17)

Element	p.p.m. Dry weight	Element	p.p.m. Dry weight
Al	35.0	As	1.6
B	21.0	Ba	4.1
Br	25.0	Ca	39,000.0
Cl	4,500.0	Co	0.052
Cr	0.42	Cu	4.4
Fe	160.0	Ga	0.064
I	0.27	K	34,000.0
Mg	1,500.0	Mn	13.0
Mo	3.7	N	43,000.0
Na	2, 600.0	P	2,200.0
Pb	1.6	Rb	56.0
Ru	0.0045	S	17,000,0
Se	0.17	Si	240.0
Sr	75. 0	Ti	$\leq$ 0.3
V	$\leq$ 0.2	W	0.04
Zn	30.0		

TABLE B.10.

Analysis of Standard Solutions of Antimony.

Theoretical Concentration (μg/ml)	Found (μg/ml)	Error (%age of theoretical)
0.250	0.264	+ 6.0
0.125	0.125	0.0
0.0675	0.0678	+ 0.4
0.03875	0.0380	- 2.0
0.00675	0.00620	- 8.0

TABLE B.11

Calculated Activities Induced in the Halogens at a Thermal Neutron Flux of 10^{12} n/cm^2/sec., after a 30 minute Irradiation, After Bowen (18).

Product Nuclide	Half-life	Disintegrations/min/10^{-9}g
F^{20}	11 sec	17
Cl^{38}	37 min	62
Br^{80m}	4.6 hours	49
Br^{80}	18 min	140
I^{128}	25 min	980

TABLE B.12.

Principal Emissions of the Neutron Capture Activation Products of the Halogens.

Nuclide	Beta Energies (MeV)	Gamma Energies (MeV)
Cl^{38}	1.11 (31%) 2.77 (16%) 4.81 (53%)	1.60 (31%) 2.15 (47%)
Br^{80m}		0.04 (43%)
Br^{80}	1.4 (14%) 2.0 (78%)	0.62 (14%)
Br^{82}	0.44 (100%)	0.55 (75%) 0.62 (42%) 0.70 (28%) 0.78 (83%) 0.83 (25%) 1.04 (29%) 1.32 (28%) 1.48 (17%)
I^{128}	1.67 (16%) 2.12 (76%)	0.46 (17%) 0.54 (1.8%)

Only the most abundant emissions are listed. Relative abundances are quoted in parentheses.

TABLE B.13.

The Determination of Protein Bound Iodine in Serum by Activation Analysis (Simple two stage separation).

Sample No.	Iodine Content by the Chemical Method (μg%)	Iodine Content by Activation Analysis (μg%)
1	13.6	14.85
2	16.6	15.3
3	6.2	6.8, 7.87
4	6.0	7.86
5	5.0	9.4, 9.9
6	4.8	13.03, 14.3

TABLE B.14.

Activation Analysis of Serum for Protein Bound Iodine Using the Shortened Distillation Technique.

Sample No.	Iodine Content by the Chemical Method (μg%)	Iodine Content by Activation Analysis (μg%)
3	6.2	11.0
4	6.0	7.0
5	5.0	4.8
6	4.8	3.8
7	1.3	20.0
8	2.4	7.0
9	4.3	8.0
10	4.4	10.5
11	15.1	22.9

TABLE B.15.

Neutron Capture Products of Copper, Zinc, and Cadmium, after Allen et al (2).

Product	Capture Cross-section of the natural element (barns)	Activity produced after irradiation for one half-life at a flux of 10^{12} n/cm^2/sec. (mc/g natural element)	Half-life	Useful Emissions
Cu^{66}	0.56	70.0	5.1 min	β
Cu^{64}	3.0	380.0	12.8 hrs	β, β^+
Zn^{65}	0.22	27.0	245.0 days	γ
Zn^{69m}	0.018	2.2	13.8 hrs	γ
Zn^{69}	0.186	23.0	55.0 min	β
Cd^{115m}	0.04	2.9	43.0 days	β
Cd^{115}	0.32	33.0	2.3 days	β, γ
In^{115m}	Daughter of Cd^{115}	33.0	4.5 hrs	γ
Cd^{117}	0.11	8.0	2.9 hrs	β
In^{117}	Daughter of Cd^{117}	8.0	1.1 hrs	β, γ

TABLE C.1.

Analysis of Copper Rich Serum Dried over Silica Gel Before Activation.

Sample	Copper Added (µg/ml)	Total Copper Concentration (µg/ml)	Copper found (µg/ml)
Pooled Serum	Nil	-	1.36 1.37
Serum with added Copper (A)	0.70	2.065	2.06 2.25
Serum with added Copper (B)	2.80	4.165	3.88 3.43

TABLE 6.2.

Serum Copper Levels in Families in which Wilson's Disease is Suspected.

Patient	Serum Copper (μg/ml)	Patient	Serum Copper (μg/ml)	Patient	Serum Copper (μg/ml)
W.S.	0.437	T.F.	0.39	S.W.	0.77
Brother of W.S.	0.865	R.F.	0.97	A.W. (Brother)	1.26
Father of W.S.	0.64	J.F.	1.01	Mother of S.W.	1.03
Mother of W.S.	0.79	I.F.(Brother)	1.04	Father of S.W.	0.80
		A.F.	0.78		
		I.F. (Sister)	0.27		
		E.F.	0.70		

TABLE C.3.

Analysis of Copper Rich Urine.

Sample	Copper Added (µg/ml)	Total Copper Concentration (µg/ml)	Copper Found (µg/ml)
Untreated Urine	Nil	-	0.555 0.575
Urine with added Copper (A)	0.70	1.265	1.30 1.41
Urine with added Copper (B)	2.80	3.365	3.26 3.12

TABLE C.4.

Normal Urinary Copper Content and Daily Excretion of Copper (Orthopaedic Patients).

No.	24 hr. vol. (ml)	Copper content (µg/ml)	Daily Copper Excretion (mg/24 hr)
1	950.0	0.122	0.12
2	2450.0	0.048	0.12
3	560.0	0.155	0.09
4	1020.0	0.051	0.05
5	1500.0	0.042	0.06
6	790.0	0.040	0.03
7	1750.0	0.068	0.12
8	2920.0	0.112	0.33
9	650.0	0.223	0.15
10	-	0.125	-
11	-	0.079	-

TABLE C.5.

Urinary Copper Content in Patients with Normal Copper Metabolism treated with D penicillamine.

Case No.	Date of Sample	Copper Content (µg/ml)
1.	12-13/10/63	0.500
	19-20/10/63	0.210
	23/5/64	0.270
2.	12-13/10/63	0.620
	29-30/11/65	0.030
3.	23-24/1/64	0.004
	29/2 - 1/3/64	0.035

TABLE C.6.

Urinary Content and Daily Excretion of Copper in Cases of Wilson's Disease, under a Regimen of Penicillamine.

Date	Total Urine Vol. (24 hrs) (ml)	Treatment	Copper Content (µg/ml)	Daily Copper Excreted (mg)
Case 1, W.S.				
16-17/9/64	2240.0		0.23	0.52
22-23/9/64	690.0	+	2.10	1.45
23-24/9/64	1050.0	+	0.91	0.96
24-25/9/64	1290.0	+	1.40	1.81
25-26/9/64	1230.0	+	2.07	2.55
27-28/9/64	2580.0	+	0.32	0.83
28-29/9/64	1210.0	+	2.37	2.87
2-3/10/64	1120.0	+	1.47	1.65
4-5/10/64	1020.0	+	0.44	0.45
6-7/10/64	1110.0	+	0.53	0.59
10-11/10/64	510.0	+	2.54	1.29
12-13/10/64	1160.0	+	0.80	0.93
14-15/1/65	1520.0	+	0.55	0.94
15-16/1/65	710.0	+	0.46	0.33
16-17/1/65	900.0	+	0.69	0.62
17-18/1/65	1260.0	+	0.35	0.44
22-23/1/65	1220.0	+	0.37	0.45
Case 2, T.F.				
28-29/5/65			0.41	-
17-18/6/65	2650.0	+	0.30	0.785
18-19/6/65	2020.0	+	1.63	3.28
19-20/6/65	1720.0	+	1.32	2.26
20-21/6/65	2500.0	+	0.61	1.52
21-22/6/65	2500.0	+	1.26	3.14
22-23/6/65	2450.0	+	1.22	2.96
23-24/6/65	2000.0	+	1.90	3.80
24-25/6/65	1740.0	+	1.15	2.01
Case 3, I.F., sister of T.F.				
26-27/10/65		+	1.24	-
27-28/10/65		+	2.20	-
20-21/11/65	740.0	+	0.33	0.24
21-22/11/65	400.0	+	0.45	0.18
Case 4, S.W.				
21-22/6/65	1960.0	+	0.845	1.65
23-24/6/65	1780.0	+	1.27	2.26

TABLE C.7.

Copper Content of Hair and Nail (p.p.m.)., in Wilson's Disease, Cirrhosis, and the Normal State.

Case	Hair	Nail
T.F.,	14.8	17.7
S.W.,	22.2	9.45
I.F.,	19.1	35.4
A.W.,(Brother of S.W.)	18.1	88.1
Mother of S.W.,	25.0	17.0
Father of S.W.,	12.8	27.4
Cirrhosis of Liver	21.2	11.4
Normal Value (mean ± std. devn.)	23.1 ± 11.5	18.1 ± 12.1

Industrial Exposure, Male Workers, Mercury contents in p.p.m.

Subject	Head Hair		Body Hair		Nail		Urine	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
M.N.,	98.6	31.8	208.0	7.5	1068.0	25.0	0.36	0.12
B.D.,	34.6	5.1	60.0	1.9	20.0	6.7	0.024	0.005
J.D.,	45.5	-	32.4	-	13.1	-	0.015	-
B.E.G.,	-	2.1	-	1.2	-	0.47	-	0.001

(A) - Samples taken under working conditions.

(B) - Samples taken after the work place has been thoroughly decontaminated.

Note that B.E.G. is not employed in handling mercury, and does not show any undue contamination.

TABLE 6.9.

Normal Values for the Mercury Content of Hair, Nail, Skin, Urine, and Whole Blood. Values are given in p.p.m.

Tissue	No. of Samples.	Maximum	Minimum	Median	Mean	Std. Devn. (Linear)	Mean	Std. Devn. (Logarithmic)
Hair, head and body, male subjects.	18	9.30	0.025	1.78	2.75	2.72	1.50	4.11
Hair, head and body, female subjects.	28	72.0	0.556	3.23	10.42	18.85	4.38	3.29
Nail, both sexes.	25	33.8	0.80	4.76	7.27	8.0	4.50	2.70
Skin, both sexes.	18	18.7	0.25	2.00	3.34	4.49	1.92	2.87
Urine, both sexes.	10	0.010	0.001	-	-	-	-	-
Blood, both sexes.	Literature values for the mercury content of whole blood in p.p.m. dry weight are (a) 0.03, 0.013 (73), and (b) 0.043 (19).							

TABLE C.10.

Mercury Contamination of Dental Chair Assistants, Mercury Content of Hair and Nail. Values given in p.p.m.

Case No.	Hair		Nail	
	Head	Body	Finger	Toe
1.	4.26	3.37	6.82	5.00
2.	4.52	-	3.72	4.32
3.	12.95	1.67	31.76	2.92
4.	5.73	9.69	4.49	2.90
5.	3.55	1.22	2.78	4.95
6.	3.56	2.23	47.50	19.40
7.	195.5	-	4.78	5.63
8.	5.43	0.40	12.99	3.00
9.	18.08	3.42	67.2	13.73
10.	189.0	9.72	172.3	3.38
11.	15.30	3.06	12.45	3.06
12.	13.13	13.99	189.9	42.9
13.	15.29	8.56	40.1	21.66
14.	15.17	3.63	10.98	2.39
15.	71.25	3.01	15.01	2.75
near (Mean	40.19	4.92	41.52	9.19
near (Std. Devn.(±)	64.05	4.15	59.8	11.23
hmic (Mean	16.63	3.39	17.73	5.76
hmic (Std. Devn.(×/÷)	3.60	2.65	3.85	2.48
Median	13.13	3.37	12.99	4.33

TABLE C.11.

Urinary Mercury Content (μg/ml) during Treatment of Pink's Disease (Acrodynia) with N-acetyl-D-penicillamine.

Case 1, S.M., Day No.	Urine Mercury Content (μg/ml)	Case 2, D.M., Day No.	Urine Mercury Content (μg/ml)	Case 3, V.L., Day No.	Urine Mercury Content (μg/ml)
1.	0.205	1.	0.416	1.	0.404
1.	0.086	2.	0.505	2.	0.214
1.	0.101	3.	0.859	4.	0.684
2.	0.164	4.	0.441	5.	0.151
3.	0.264	6.	1.590	6.	0.580
4.	0.258	7.	0.940	7.	0.285
5.	0.160	8.	0.696	9.	0.366
6.	0.303	9.	0.312	10.	0.213
7.	0.720	10.	0.826	11.	0.342
8.	0.250	11.	1.302	12.	0.171
9.	0.281	13.	0.703	15.	0.336
10.	0.172	14.	0.963		
11.	0.296	15.	0.695		
12.	0.081	18.	0.519		
13.	0.048	19.	0.519		
14.	0.078	20.	0.480		
20.	0.167	22.	0.476		
178.	0.016	23.	0.701		
		48.	0.243		
Drug administered from day 3 to day 12 inclusive.		Drug administered from day 6 to day 15 inclusive.		Drug administered from day 2 to day 12 inclusive.	

TABLE C.12.

Chronic Mercury Poisoning, Mercury Content of Skin, Hair, and Nail, of the entire exposed family. Values are given in p.p.m. dry weight.

Sample	Father	Elder Son	Mother	Daughter	Younger Son
Head Hair	6.89	17.5	141.0	8.29	8.36
Body Hair	4.05	131.0	6.35	-	-
Finger Nail	36.4	-	20.2	3.6	-
Toe Nail	51.5	71.7	35.1	53.1	138.0
'Normal' Skin	11.3	92.5	-	2.00	-
Exfoliating Skin	20.9	156.0	-	-	-

TABLE C.13.

Changes in the Blood Mercury Content, in Chronic Exposure, Before and During Treatment, and after Symptoms have Reappeared in the Father and Son. Values are given in p.p.m. dry weight.

	Father	Elder Son	Mother	Daughter	Younger Son
Before Treatment	3.38	3.92	2.16	2.03	3.28
First Day of Treatment	2.54	2.35	2.28	2.30	2.40
After Symptoms Reappeared in father and elder son	0.584	0.523	0.348	-	-

TABLE C.14.

Change in the Mercury Content of Urine, during Treatment for Chronic Exposure to Mercury. Values are given in p.p.m. (μg/ml).

	Father	Elder Son	Mother	Daughter	Younger Son
Before Treatment	0.270	3.74	0.100	0.294	0.117
During Treatment (Day 1)	0.420	2.46	0.131	0.152	0.176
(Day 2)	0.620	1.16	0.123	0.173	0.193
(Day 3)	0.326	1.45	0.113	0.029	-
(Day 4)	0.340	-	0.053	-	-
(Day 5)	0.272	-	-	-	-
After Symptoms reappeared in father and elder son	0.710	0.762	0.558	0.502	0.434
Saliva Mercury content	0.018 0.027	0.099	0.008 0.007	-	-

TABLE 6.15.

Comparison of Results by Activation Analysis and Flame Photometry for the Estimation of Strontium and Calcium in a Variety of Samples.

Sample	%age Calcium		Strontium (p.p.m.)	
	Flame Phot.	Activation	Flame Phot.	Activation
Ashed Faeces	19.64	17.4	228.5	163.6
Ashed Faeces	28.13	26.7	308.0	234.0
Ashed Bone	38.0	38.5	-	97.9 107.0
Whole Bone	13.5	-	30.6	33.9
Ashed Diet	7.29	3.82 5.74 9.01	111.8	64.3 67.5
Ashed Diet	-	-	82.37	56.5 160.0
Teeth	-	-	-	83.5 52.8 69.6 62.2

Activation Analysis does not give reproducible results for either calcium or strontium in ashed diet.

TABLE C.16.

Total weekly excretion of calcium and Sr^{84} in urine and faeces, and serum levels.

Week No.	Total Faecal Extraction			Total Urinary Excretion				Serum Level	
	Ca (g)	Sr^{84} (μg)	μg Sr^{84}/g Ca	Ca (g)	Sr^{84} (μg)	μg Sr^{84}/g Ca	Ca (mg%)	Sr^{84} (μg%)	μg Sr^{84}/g Ca
1.	12.45	73.35	5.9	3.79	13.59	3.58	10.1	0.032	3.17
2.	18.3	133.0	7.25	1.642	13.17	8.00	10.2	0.030	2.94
3.	9.35	81.03	8.66	1.345	11.04	8.22	10.3	0.052	5.05
4.	8.71	55.55	6.38	1.964	12.52	6.40	10.2	0.044	4.40

TABLE C.17.

Activation Analysis Results for copper, zinc, and Manganese, content of liver and blood, in a case of haemochromatosis (H), and normal tissues (N), compared with literature values. Values are expressed in p.p.m. dry weight.

	Tissue	Copper		Zinc		Manganese	
		(H)	(N)	(H)	(N)	(H)	(N)
Activation Analysis	Liver	18.5	25.2	178.0	246.0	4.0	6.5
	Blood	2.34	10.0	63.0	37.0	0.20	0.86
Literature Values	Liver (A)	73.0	45.3	225.0	276.0	4.0	7.0
	Blood (B)	-	6.8	-	41.0	-	0.165

(A) - Values taken from Butt et al (125). (B) - Values taken from Bowen (19).

TABLE C.18.

Antimony and barium contamination on the skin of subjects having fired or handled a revolver.

	Barium (μg)	Antimony (μg)
E.R., having fired one shot	0.084	0.003
E.R., having fired two shots	0.137	0.0221
H.S., handled gun after one shot	0.082	0.008
H.S., handled gun after two shots	0.106	0.178

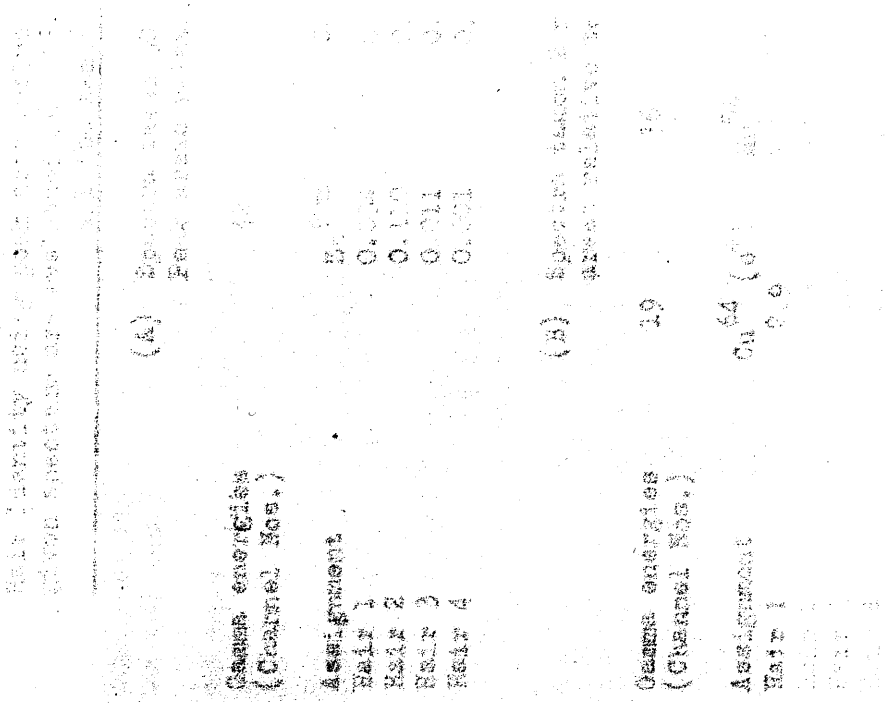


TABLE C.19.

Hair Identity using peak area evaluation. The areas of all the peaks appearing in a given spectrum are evaluated as multiples of a selected peak present in the spectra of all the samples.

(A) Spectra taken 30 hrs. after the end of a 24 hr. irradiation. Peak areas related to that of the 1.37 MeV gamma ray of Na^{24}.

Gamma energies (Channel Nos.)	42	54	87	155
Assignment	Zn^{69m}	$Cu^{64}(e^+)$	Mn^{56}	Na^{24}
Hair 1	0.034	0.201	-	1.0
Hair 2	0.110	0.110	-	1.0
Hair 3	0.011	0.106	-	1.0
Hair 4	0.001	0.272	0.100	1.0

(B) Spectra taken 2 min. after end of 30 min. irradiation. Peak areas relative to 1.60 MeV peak of Cl^{38}.

Gamma energies (Channel Nos.)	19	36	60	72	96	123
Assignment	$Cu^{64}(e^+)$	Mn^{56}	Na^{24}	Cl^{38}	Cl^{38}	Na^{24}
Hair 1	2.9	1.1	1.7	1.0	1.1	0.27
Hair 2	3.3	0.8	1.1	1.0	0.8	0.23
Hair 3	0.44	0.20	1.0	1.0	1.0	0.12
Hair 4	1.1	0.25	0.85	1.0	0.9	0.16

TABLE C.19 (Continued)

(C) Spectra taken 17 min. after 30 min. irradiation. Peak areas relative to 1.60 MeV peak of Cl^{38}.

Gamma energies (Channel Nos.)	19	36	60	72	96	123
Assignment	Cu^{64} (e^+)	Mn^{56}	Na^{24}	Cl^{38}	Cl^{38}	Na^{24}
Hair 1	1.9	1.83	1.4	1.0	1.0	0.42
Hair 2	3.7	1.3	1.7	1.0	1.3	0.60
Hair 3	0.43	0.46	1.4	1.0	1.1	0.35
Hair 4	0.76	0.30	1.0	1.0	1.0	0.19

Note:- Hair 1 is from the scene of a crime, hairs 2 and 3 are from suspects, and hair 4 is an additional sample, included to assist the evaluation of the technique.

TABLE C.20.

Normal Values for the concentrations of antimony, arsenic, copper, and mercury in human tissue. Values quoted are p.p.m. dry weight.

(A) Normal Antimony Content.

Tissue	No. of Samples	Maximum	Minimum	Median	Mean (Linear)	Std. Devn.	Mean (Logarithmic)	Std. Devn.
Adrenal	18	0.694	0.016	0.098	0.151	0.182	0.091	2.72
Aorta	21	0.780	0.010	0.066	0.132	0.166	0.082	2.65
Bone	17	1.980	0.032	0.161	0.298	0.452	0.178	2.57
Brain	25	0.310	0.020	0.070	0.122	0.092	0.078	2.45
Hair	58	6.58	0.080	0.345	0.687	0.979	0.409	2.59
Heart	29	0.560	0.010	0.060	0.086	0.108	0.052	2.75
Kidney	25	1.97	0.010	0.080	0.173	0.386	0.069	3.45
Liver	27	0.35	0.020	0.110	0.138	0.093	0.107	2.17
Lung	34	2.26	0.080	0.390	0.558	0.569	0.362	2.55
Nail	11	0.560	0.017	0.080	0.175	0.191	0.103	3.03
Ovary	10	0.136	0.019	0.069	0.070	0.303	0.055	2.12
Pancreas	26	0.710	0.016	0.092	0.156	0.176	0.095	2.78
Pectoral Muscle	18	1.739	0.022	0.080	0.193	0.394	0.089	3.13
Prostate	7	1.620	0.030	0.182	0.420	0.559	0.198	4.07
Skin	10	4.40	0.014	0.065	0.618	1.357	0.112	6.72
Spleen	25	0.210	0.020	0.060	0.069	0.056	0.051	2.25
Stomach	28	0.270	0.010	0.045	0.075	0.066	0.053	2.36
Thyroid	24	0.472	0.012	0.097	0.146	0.136	0.092	2.90
Uterus	9	0.420	0.020	0.109	0.162	0.137	0.115	2.53

(B) Normal Arsenic Content.

Tissue	No. of Samples	Maximum	Minimum	Median	Mean (Linear)	Std. Devn. (Linear)	Mean (Logarithmic)	Std. Devn. (Logarithmic)
Adrenal	22	0.293	0.002	0.029	0.060	0.076	0.028	3.90
Aorta	29	0.570	0.003	0.031	0.063	0.104	0.034	2.85
Blood	12	0.920	0.001	0.038	0.147	0.269	0.035	7.04
Bone	20	0.240	0.010	0.057	0.080	0.068	0.052	2.74
Brain	19	0.036	0.001	0.013	0.015	0.009	0.012	2.30
Heart	23	0.078	0.002	0.024	0.030	0.022	0.020	2.69
Kidney	25	0.363	0.002	0.033	0.050	0.074	0.025	3.45
Liver	27	0.246	0.005	0.028	0.056	0.060	0.034	2.84
Lung	45	0.514	0.006	0.081	0.108	0.103	0.073	2.58
Menstrual Blood	22	0.462	0.011	0.072	0.087	0.095	0.060	2.36
Ovary	13	0.260	0.013	0.037	0.070	0.070	0.047	2.47
Pancreas	30	0.410	0.005	0.045	0.087	0.110	0.046	3.16
Pectoral Muscle	24	0.413	0.012	0.063	0.090	0.097	0.061	2.38
Prostate	10	0.090	0.010	0.046	0.044	0.022	0.039	1.85
Spleen	23	0.132	0.001	0.020	0.031	0.035	0.017	3.62
Stomach	21	0.104	0.003	0.037	0.037	0.034	0.021	3.28
Thyroid	22	0.314	0.001	0.042	0.078	0.085	0.042	3.88
Uterus	23	0.188	0.010	0.031	0.058	0.056	0.036	2.67

TABLE C.20. (Continued)

(C) Normal Copper Content.

Tissue	No. of Samples	Maximum	Minimum	Median	Mean (Linear)	Std. Devn. (Linear)	Mean (Logarithmic)	Std. Devn. (Logarithmic)
Adrenal	18	28.9	1.14	5.34	7.36	6.94	5.34	2.24
Aorta	25	21.9	2.4	6.3	6.68	3.93	5.93	1.61
Bone	18	11.8	0.85	2.94	4.24	3.42	3.17	2.19
Brain	25	39.4	13.1	23.2	23.89	6.35	23.11	1.30
Hair	29	54.5	7.6	19.1	23.09	11.68	20.64	1.61
Heart	24	22.9	10.1	16.3	16.48	3.68	16.07	1.26
Kidney	23	35.7	5.10	13.2	14.90	6.89	13.72	1.50
Liver	24	46.8	9.2	25.2	25.54	11.5	22.91	1.63
Lung	21	15.9	4.2	9.5	9.54	3.55	8.90	1.47
Nail	33	58.2	3.18	14.9	18.08	12.07	14.69	1.96
Ovary	11	16.5	3.1	7.0	8.12	4.17	7.24	1.65
Pancreas	29	20.0	2.4	5.79	7.38	4.64	6.21	1.80
Pectoral Muscle	22	13.8	1.95	4.93	5.43	2.81	4.89	1.58
Prostate	9	11.0	1.76	6.58	6.47	2.79	5.8	1.72
Skin	10	5.4	0.29	1.76	1.98	1.69	1.30	2.87
Spleen	24	16.1	3.13	6.08	6.82	2.84	6.37	1.43
Stomach	20	36.6	4.5	11.0	12.55	7.69	10.84	1.71
Thyroid	24	17.5	1.63	5.37	6.05	3.22	5.39	1.62
Uterus	13	25.2	3.47	7.05	8.44	6.02	7.15	1.74

TABLE C.20.(Continued)

(D) Normal Mercury Content.

Tissue	No. of Samples	Maximum	Minimum	Median	Mean (Linear)	Std. Devn. (Linear)	Mean (Logarithmic)	Std. Devn. (Logarithmic)
Adrenal	18	2.44	0.15	0.51	0.80	0.747	0.54	2.51
Aorta	23	7.3	0.10	0.90	1.39	1.60	0.79	3.13
Bone	16	1.04	0.04	0.30	0.45	0.38	0.26	3.35
Brain	21	15.22	0.12	1.07	2.93	4.09	1.38	3.51
Hair (female)	28	72.0	0.556	3.23	10.42	18.85	4.38	3.28
Hair (male)	18	9.30	0.025	1.87	2.75	2.71	1.49	4.10
Hair (both sexes)	74	72.0	0.025	4.39	8.41	13.48	4.07	3.53
Heart	22	5.62	0.14	0.89	1.76	2.40	0.94	3.04
Kidney	20	79.3	0.08	1.51	9.03	20.16	2.19	5.05
Liver	22	20.0	0.15	1.40	3.66	4.96	1.50	4.25
Lung	21	10.5	0.3	1.33	2.54	2.69	1.51	2.89
Nail	25	33.8	0.8	4.76	7.27	8.38	4.50	2.69
Ovary	10	13.54	0.06	0.73	2.13	4.09	0.68	4.85
Pancreas	26	7.23	0.03	0.72	1.14	1.53	0.55	3.76
Pectoral Muscle	15	3.40	0.04	0.29	0.71	0.94	0.34	3.55
Skin	18	18.71	0.25	2.00	3.33	4.48	1.92	2.86
Spleen	21	7.41	0.10	0.68	1.50	1.92	0.83	2.95
Stomach	21	13.34	0.06	0.89	2.27	3.51	0.89	4.26
Thyroid	24	24.55	0.10	1.07	3.37	6.23	1.11	4.33
Uterus	11	4.3	0.13	0.87	1.42	1.47	0.69	3.98

TABLE C.21.

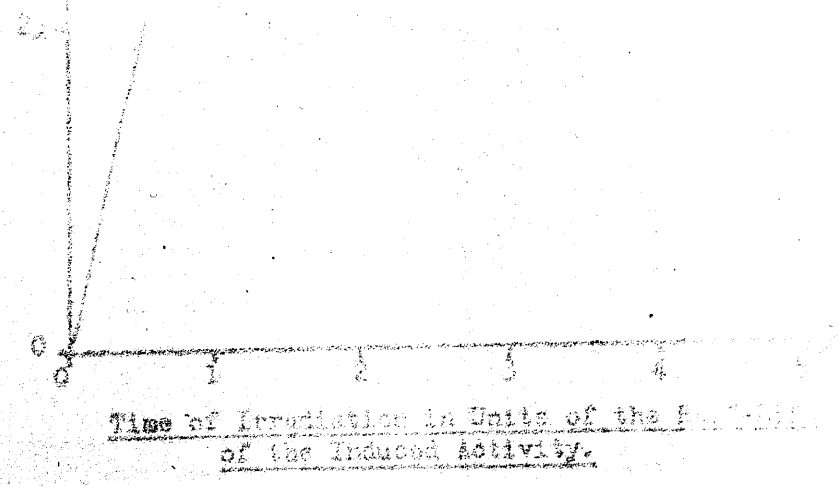
Normal Values for the Mercury Content of Human Tissues, Literature Values.

Tissue	Literature Value	Reference
Kidney	20.5 p.p.m. dry weight	(57)
	0.1 to 0.3 p.p.m. wet weight	(40)
	Average value of 0.16 p.p.m. wet weight	(148)
	0.04 to 0.10 p.p.m. wet weight, up to 127 p.p.m.	(46)
Liver	0.05 to 0.2 p.p.m. wet weight	(40)
	0.01 to 0.12 p.p.m. wet weight, up tol7.2 p.p.m.	(46)
	3.7 p.p.m. dry weight	(57)
Pituitary	0.04 to 0.13 p.p.m. wet weight	(148)
Spleen	1.2 p.p.m. dry weight	(57)
Other Tissues	0.001 to 0.01 p.p.m. wet weight	(148)

TABLE C.22.

Median Values in p.p.m. dry weight in analyses for several trace elements in carcinoma of the lung.

Element	Carcinoma	Host Tissue	Un-involved Lung.
Arsenic	0.05	0.12	0.10
Antimony	0.01	0.19	0.22
Copper	10.4	12.6	11.6
Manganese	0.34	1.25	1.09
Mercury	1.60	4.42	3.66



Time of Irradiation in Units of the
of the Induced Activity.

Figure A.1. Growth Curve for Activation.

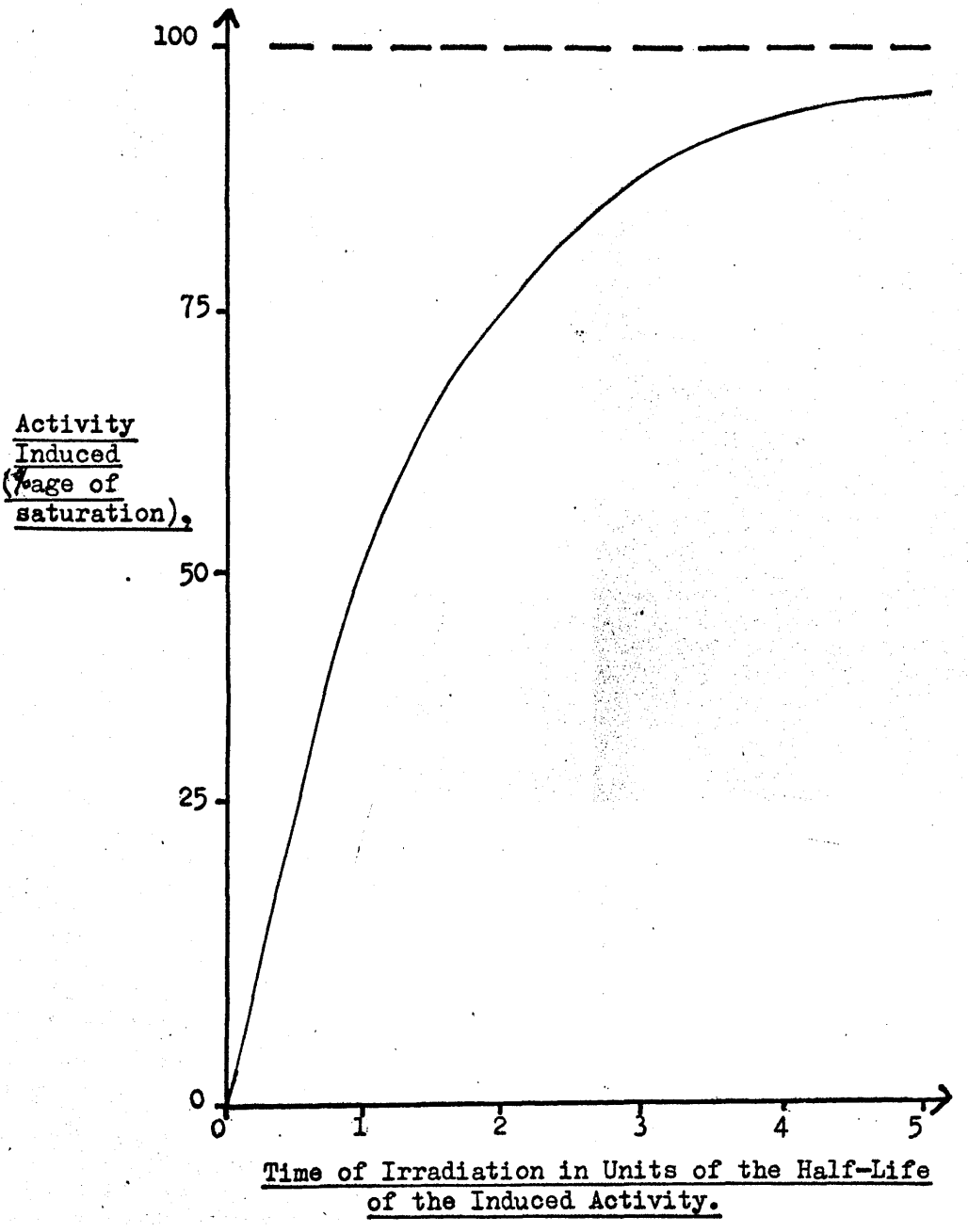


Figure A.2. Gamma Spectrometry - Block Diagram of Typical Apparatus.

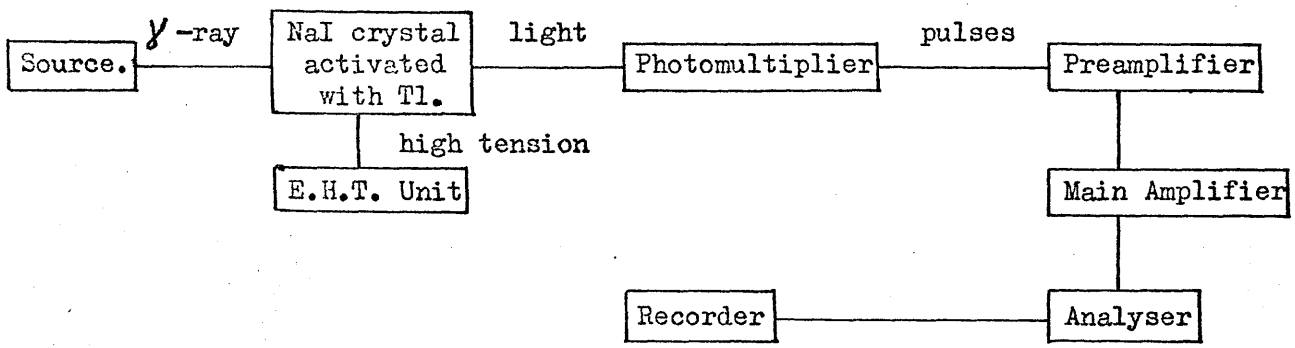


Figure A.3. Typical Output from a Spectrometer Amplifier.

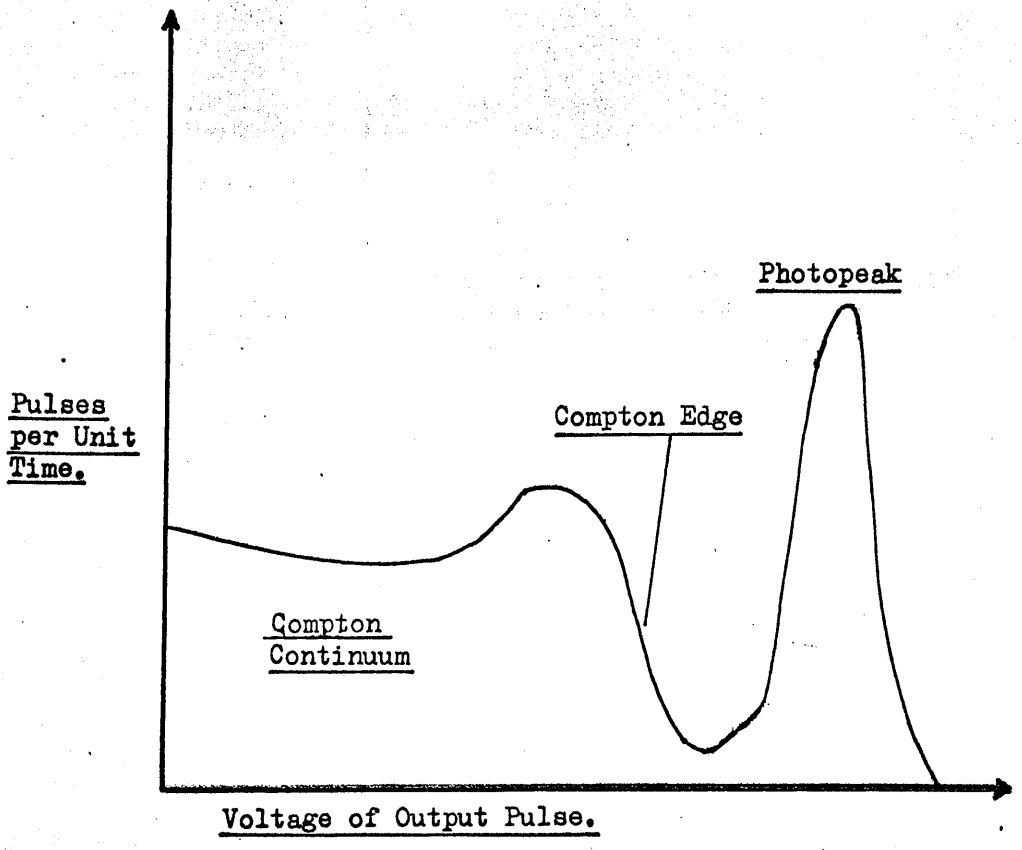
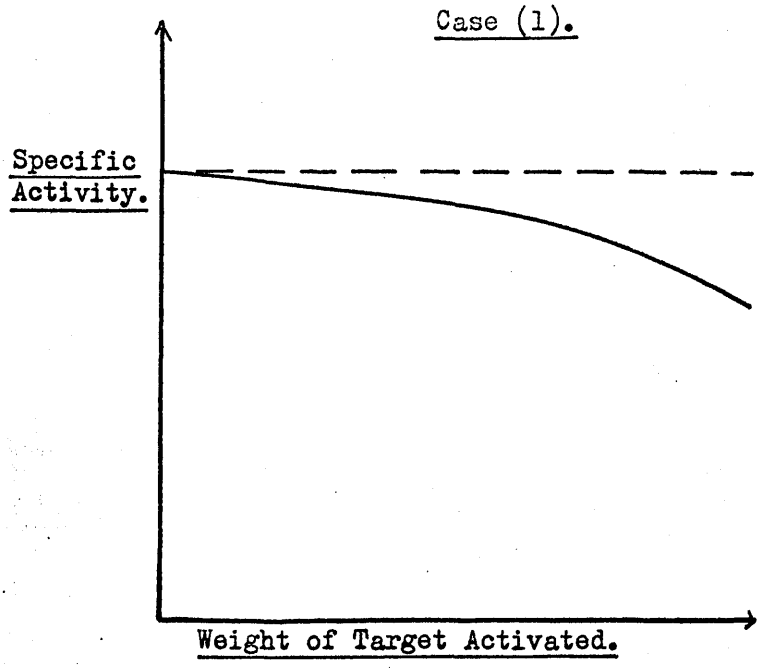
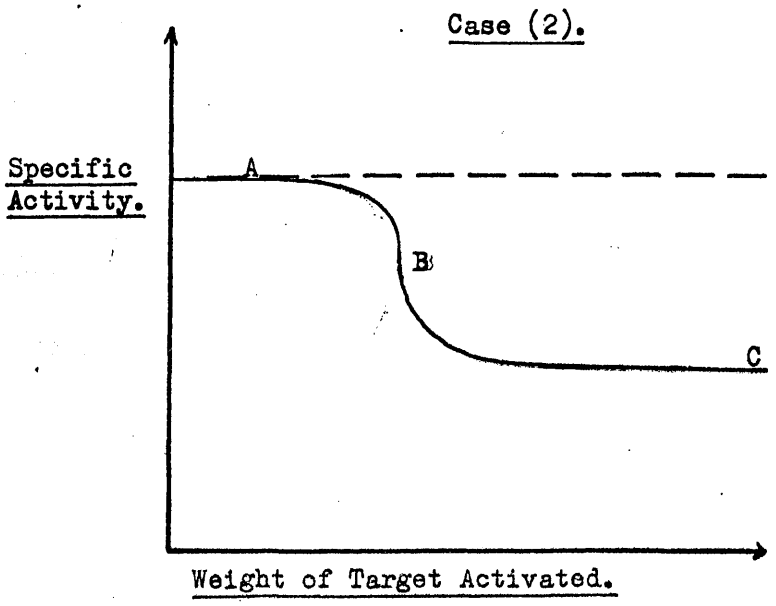


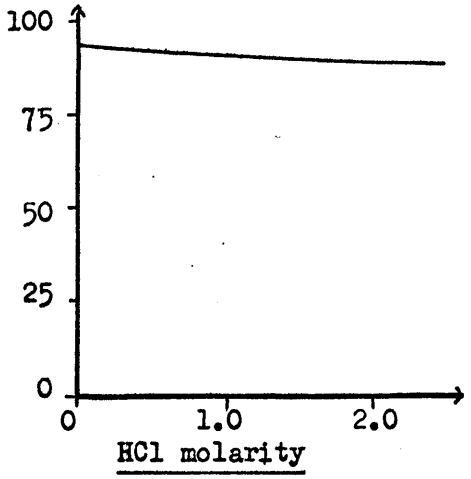
Figure A.4. Self Shielding - (1) Simple Case, and (2) in the Case of Solid Samples in Cylindrical Containers.

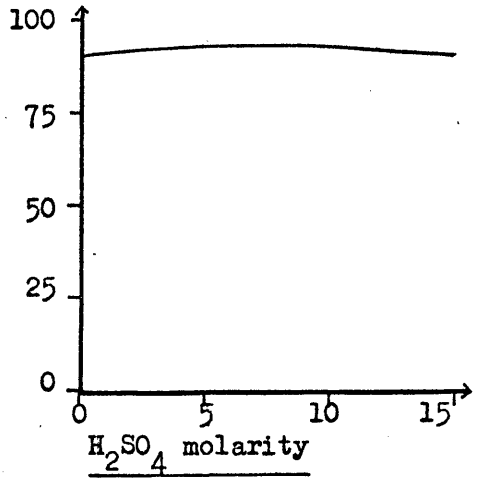


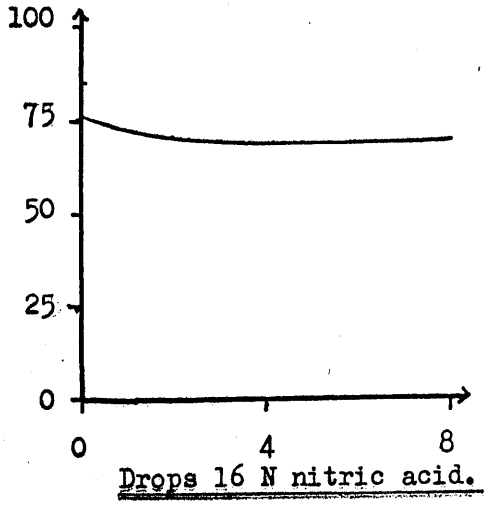


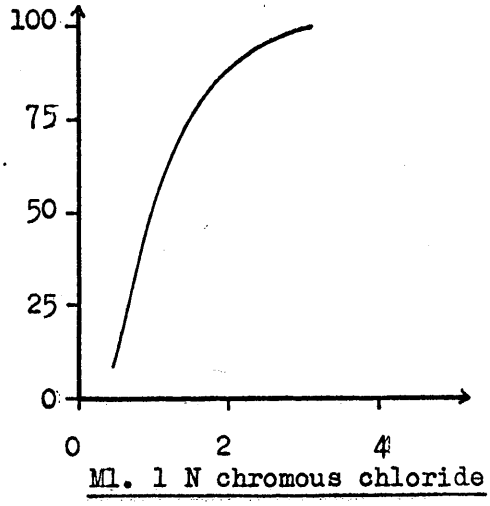
A - B, as in simple case.
B - C, specific activity becomes constant with further increase in target weight, as the induced activity depends now on the length of target.

Figure B.1. Factors Affecting the Precipitation of Antimony by Chromous Chloride.
(all abscissae %age Sb precipitated).









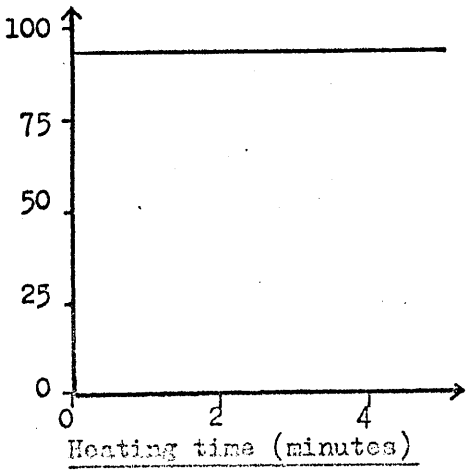
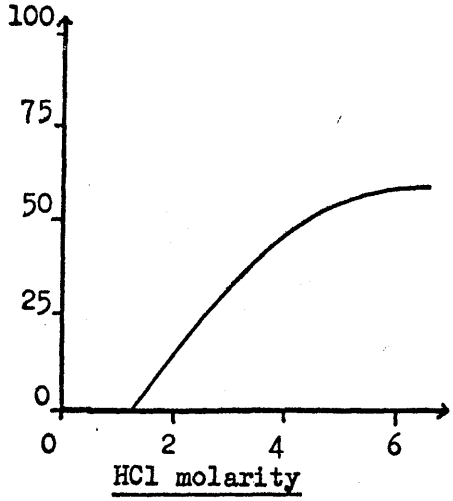
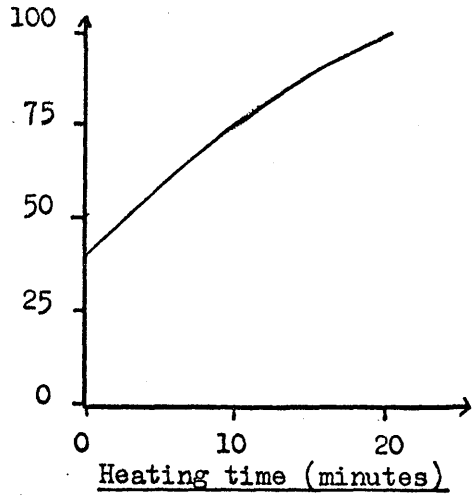


Figure B.2. Factors Affecting the Precipitation of Arsenic by Hypophosphite.
(all abscissae %age As precipitated).





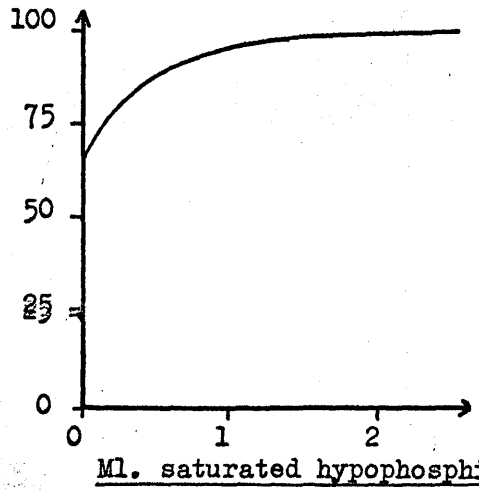
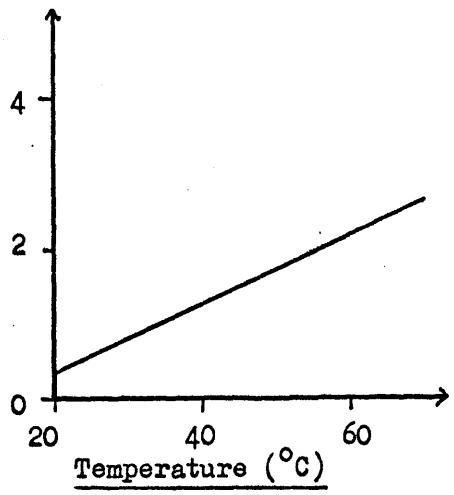
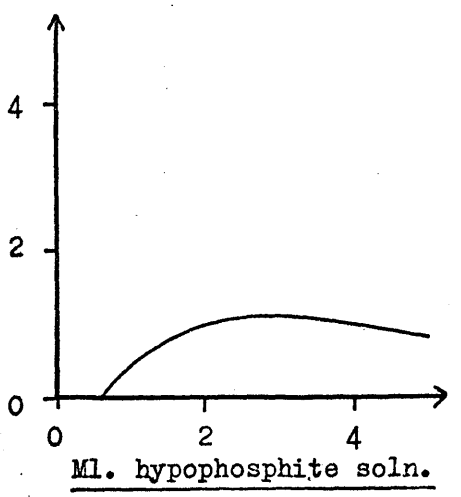
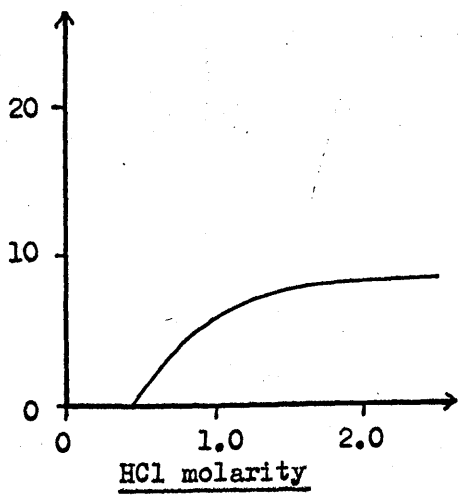


Figure B.3. Factors Affecting the Precipitation of Copper by Ferricyanide.
(all abscissae %age Cu not precipitated).







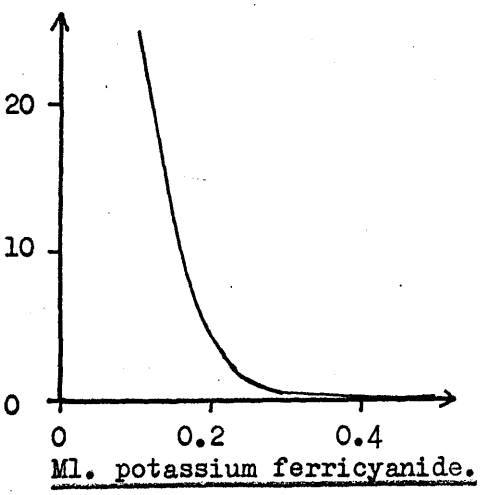


Figure B.4. Beta and Gamma Decay of Samples Analysed for Antimony.

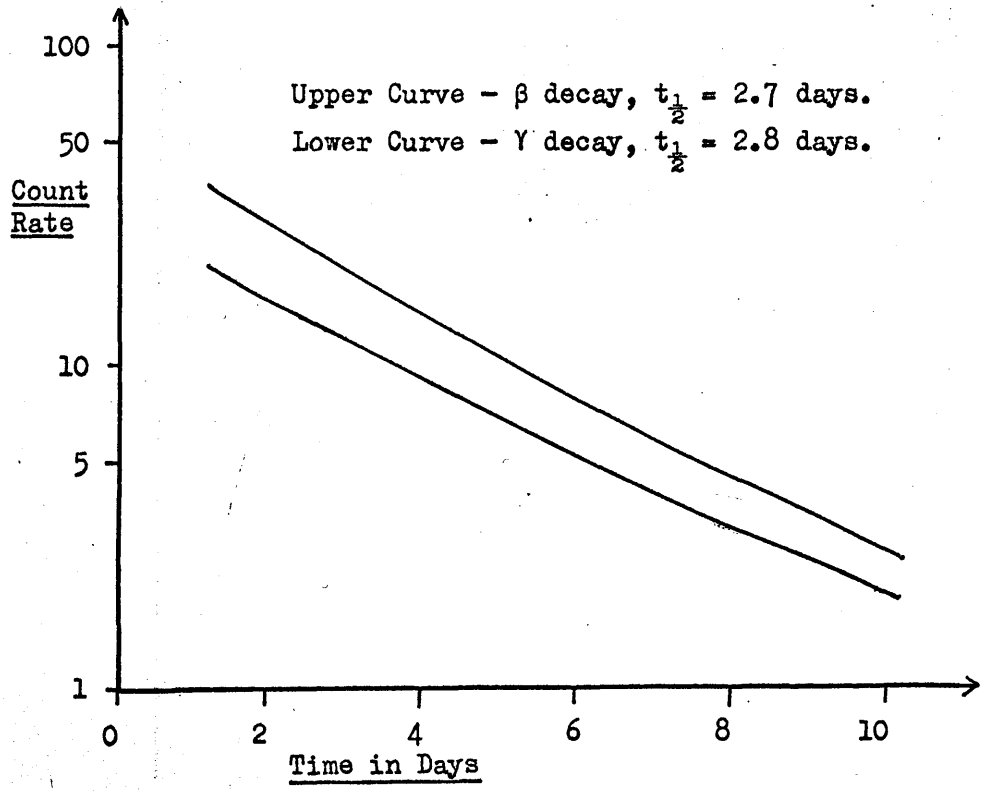


Figure B.5. Beta Decay Curves of I^{128} Separated from Serum and Ammonium Iodide.

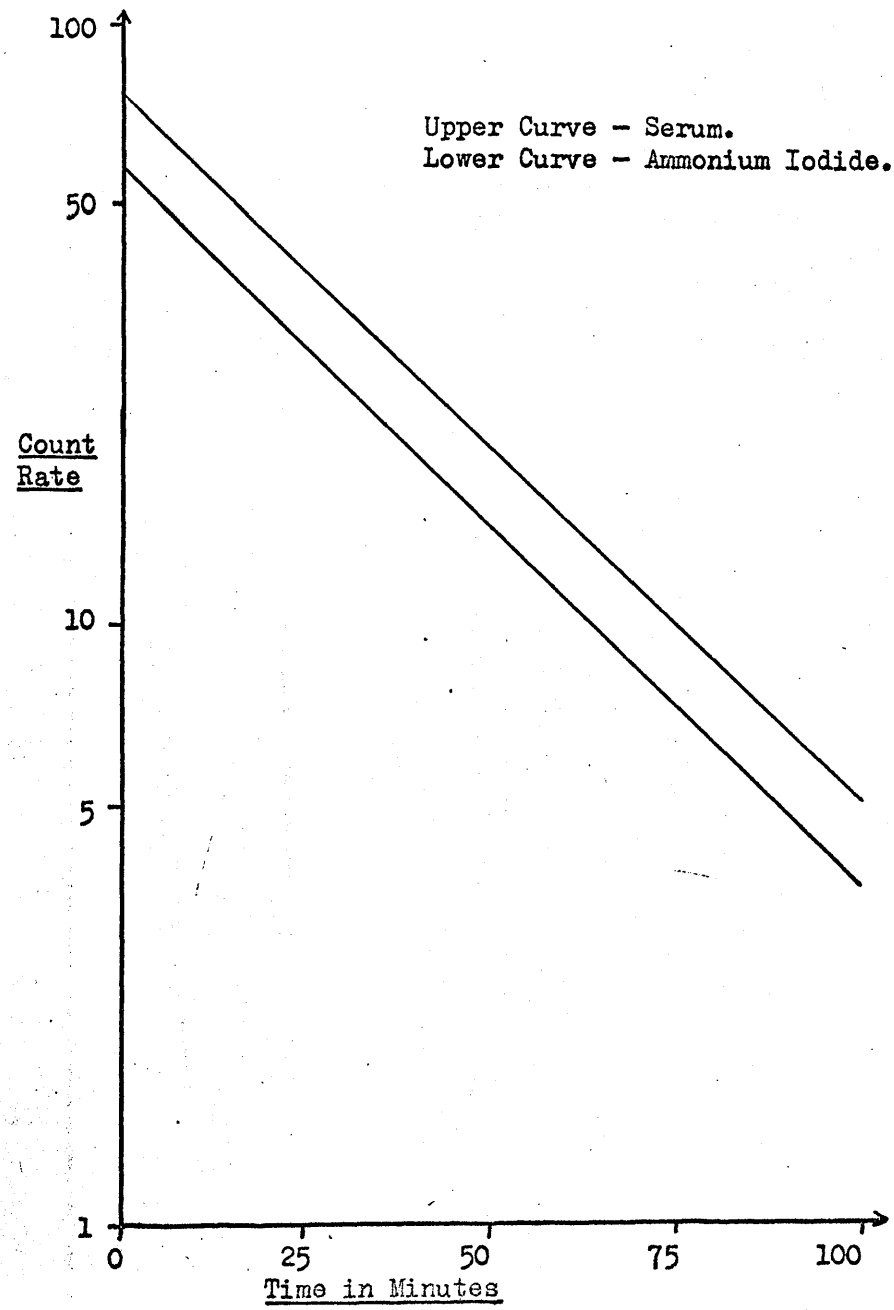


Figure B.6. Gamma Spectrum of I^{128} isolated from Serum and Ammonium Iodide.

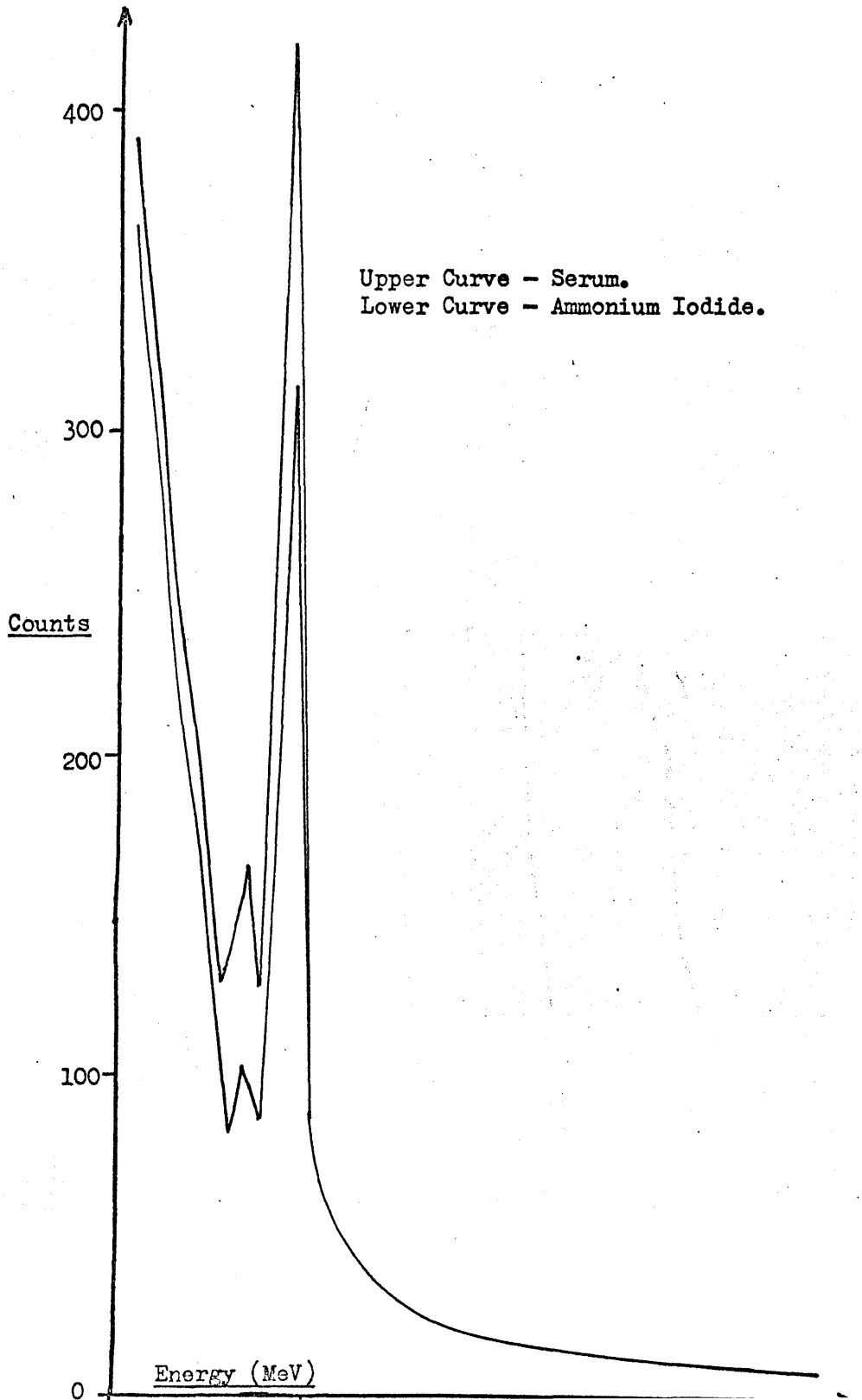


Figure B.7. Gamma Spectrum of Iodine Fraction from Ammonium Iodide activated for 3 days.

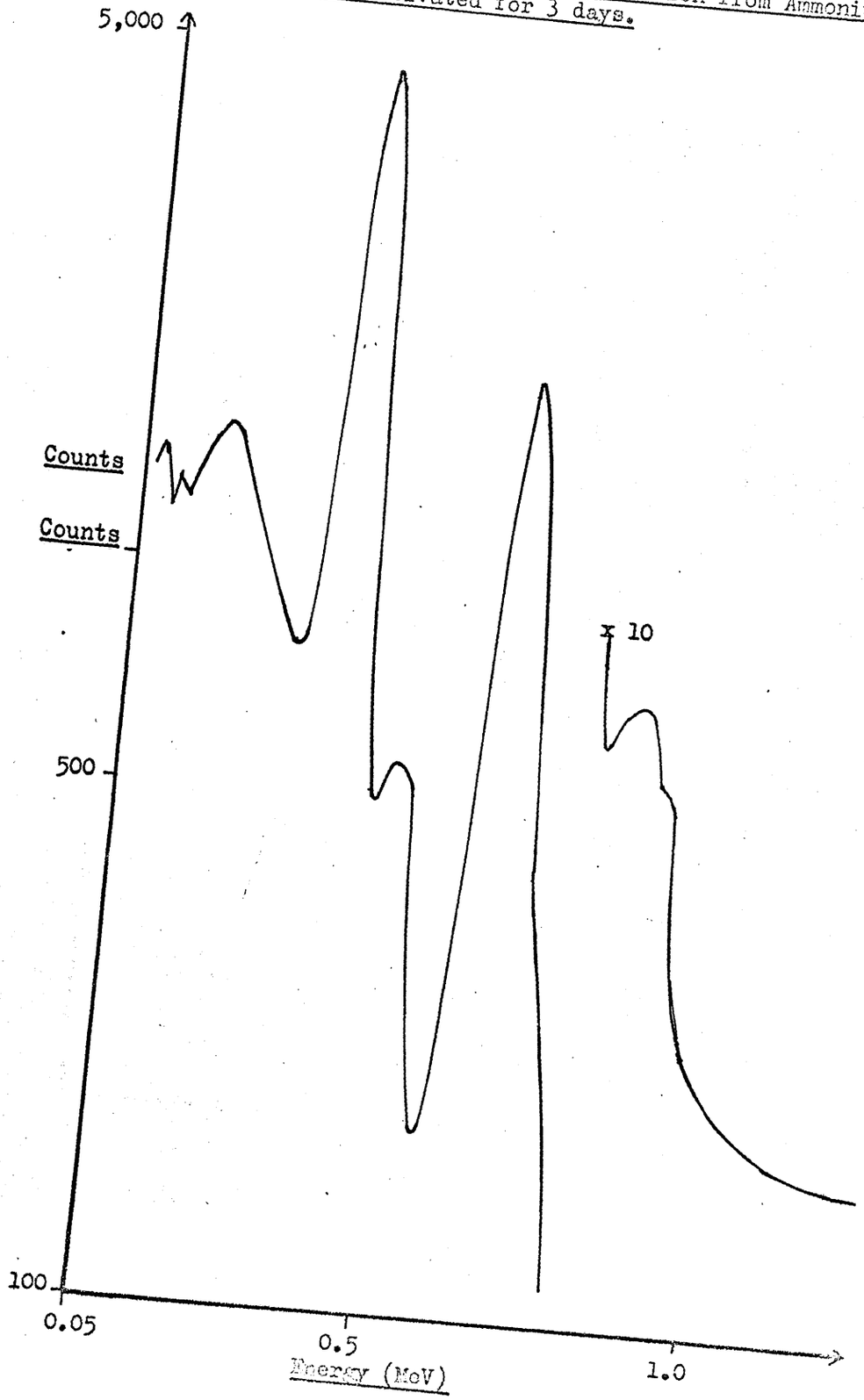


Figure B.8. Gamma Spectrum of Supernatant of Iodine Extraction.

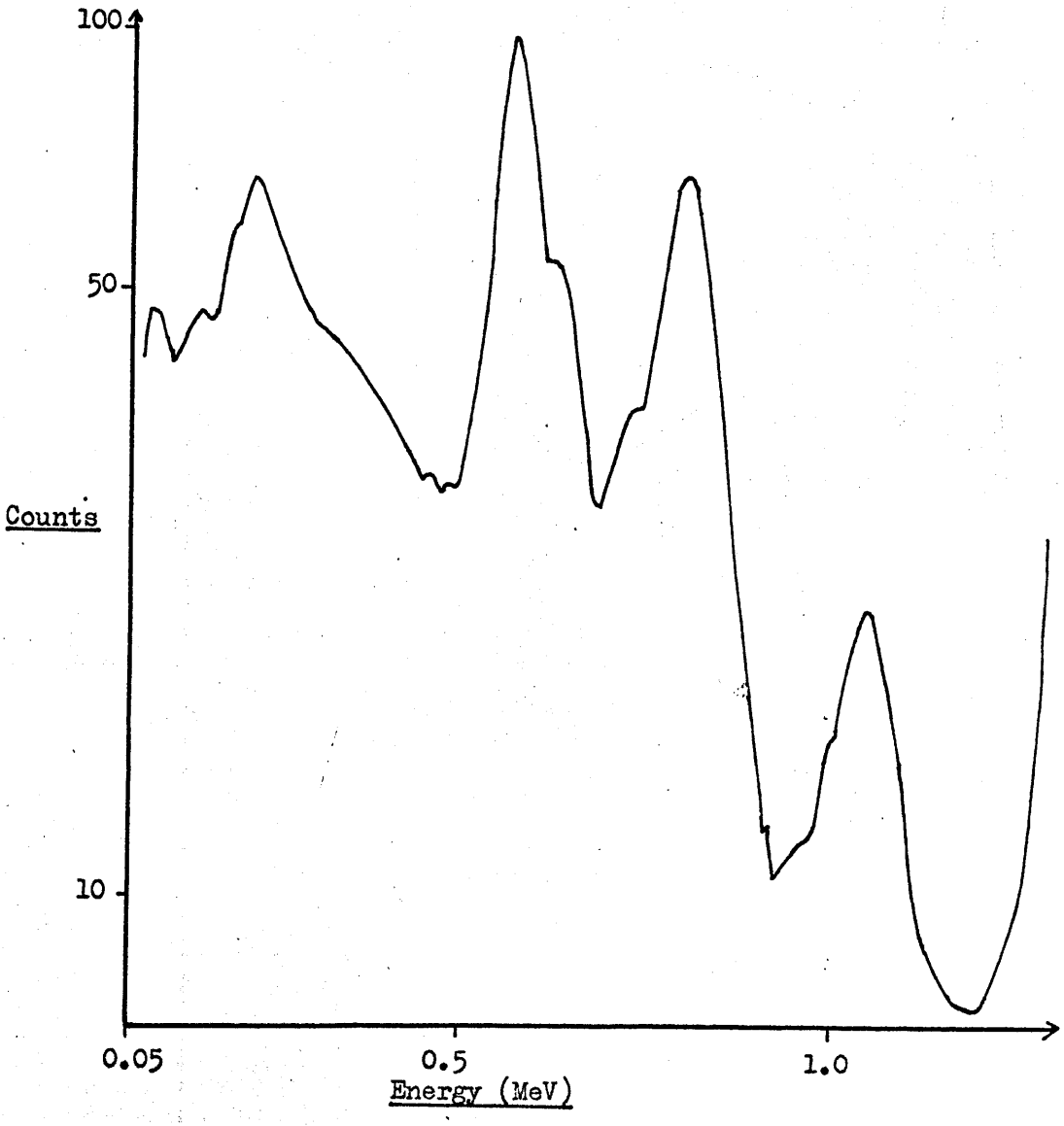


Figure B.9. Gamma Spectrum of Zinc isolated from Tooth.

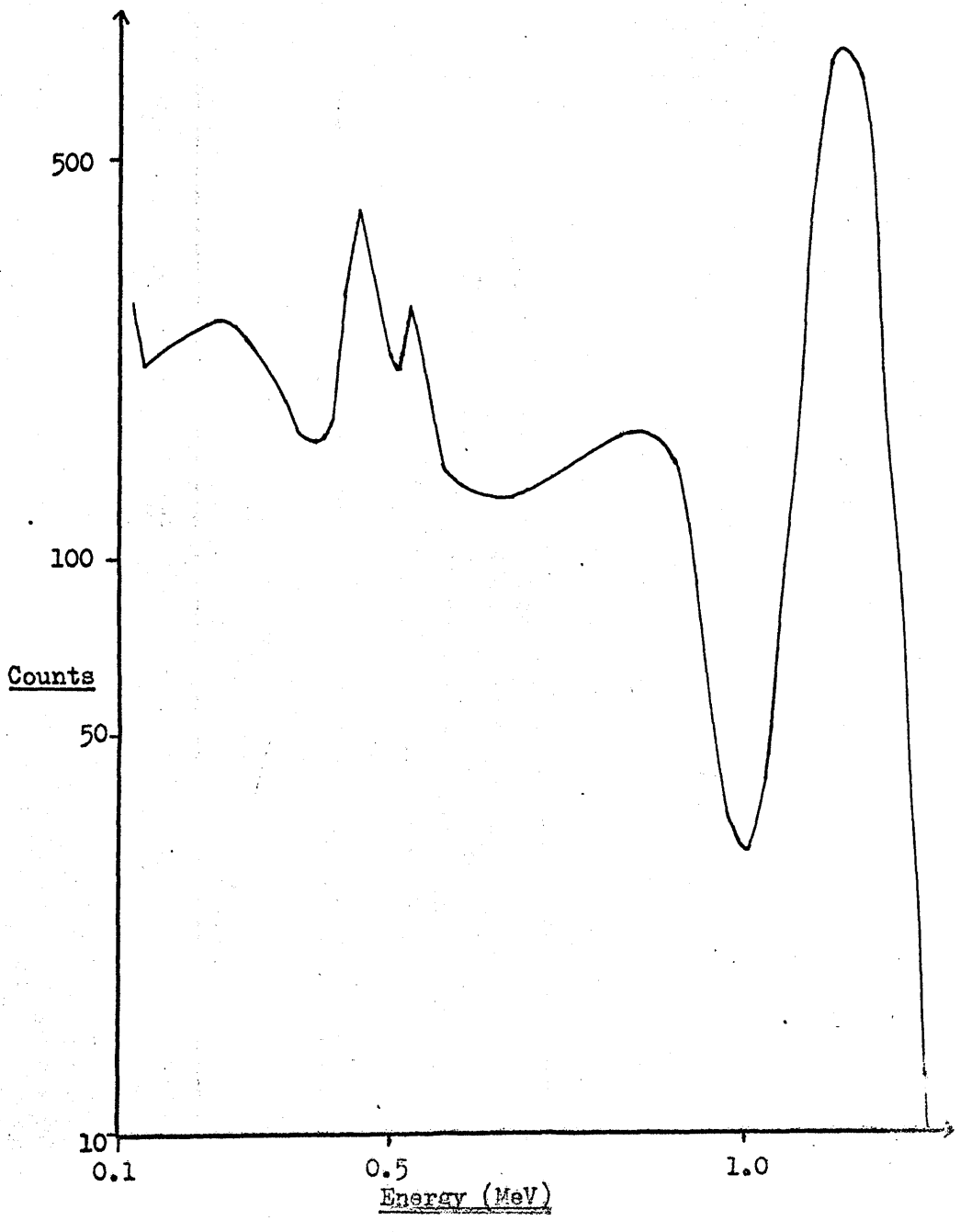
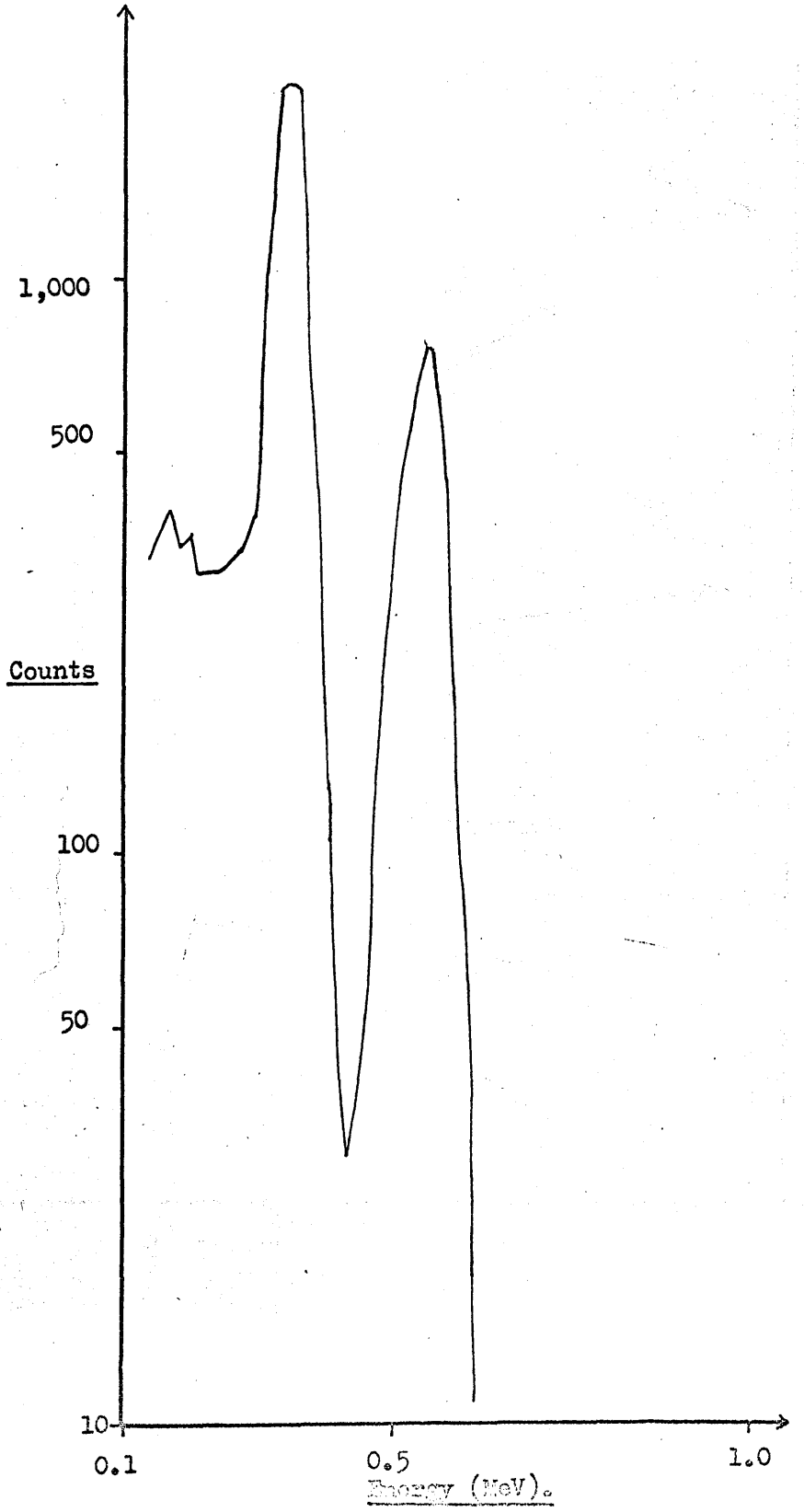


Figure B.10. Gamma Spectrum of Cadmium isolated from Kidney.



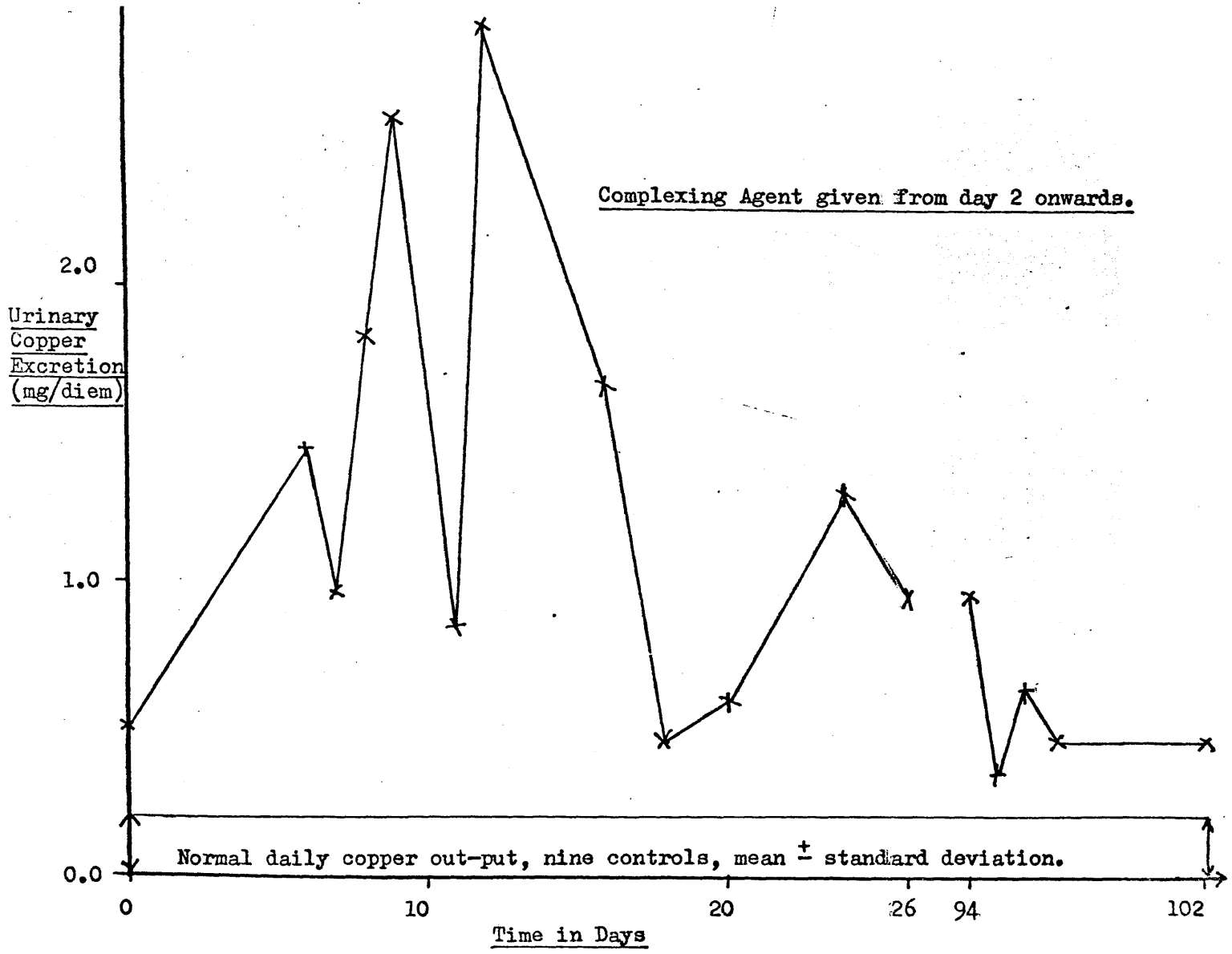

Complexing Agent given from day 2 onwards.
Urinary Copper Excretion (mg/diem)
2.0
1.0
0.0
Normal daily copper out-put, nine controls, mean ± standard deviation.
0
10
20
26
94
102
Time in Days

Figure C.2. Urine Mercury content during Treatment with N-acetyl-D-penicillamine. (Case of D.M.).

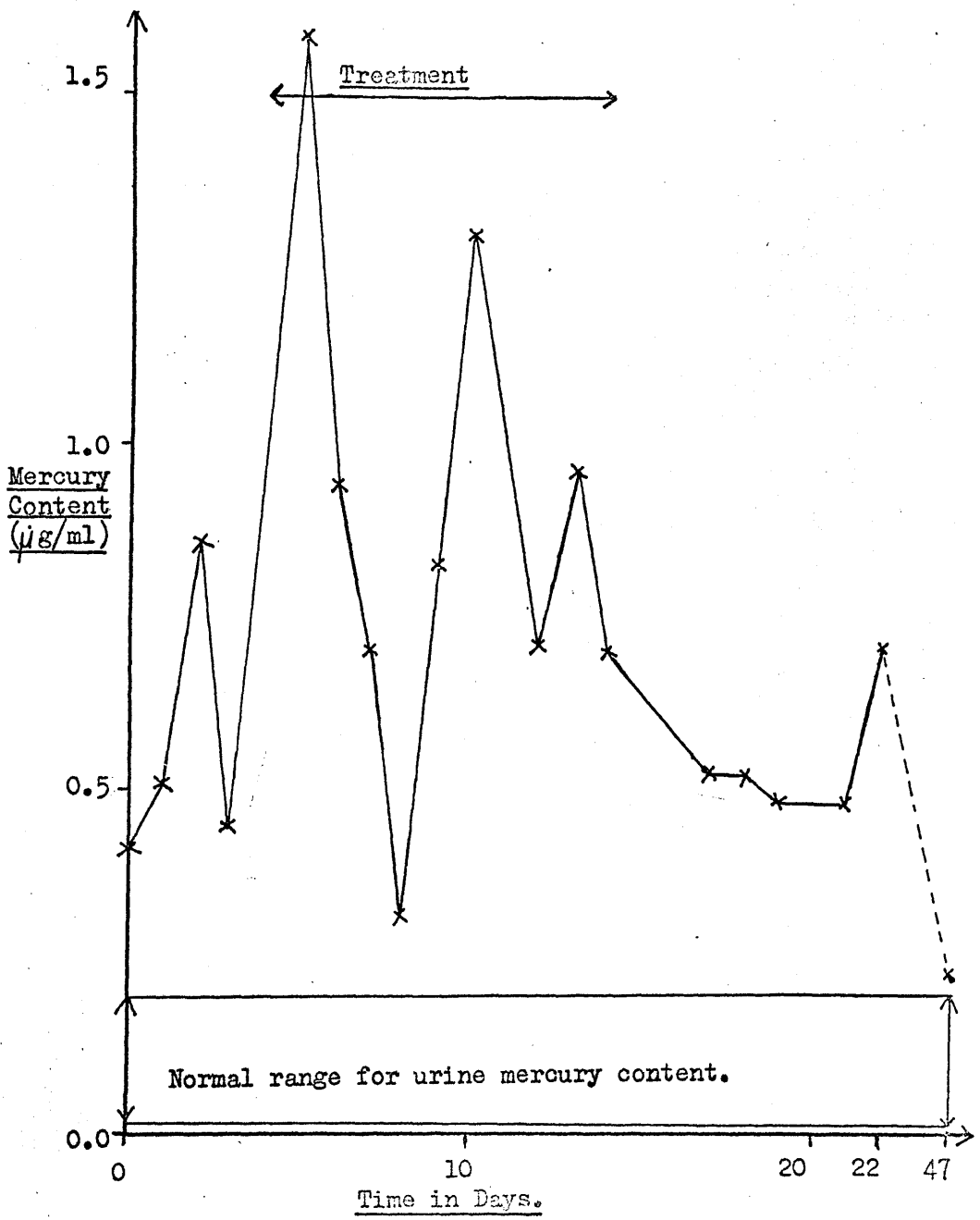


Figure C.3. Gamma Spectrum of Hair taken 2 minutes after 30 minutes activation.

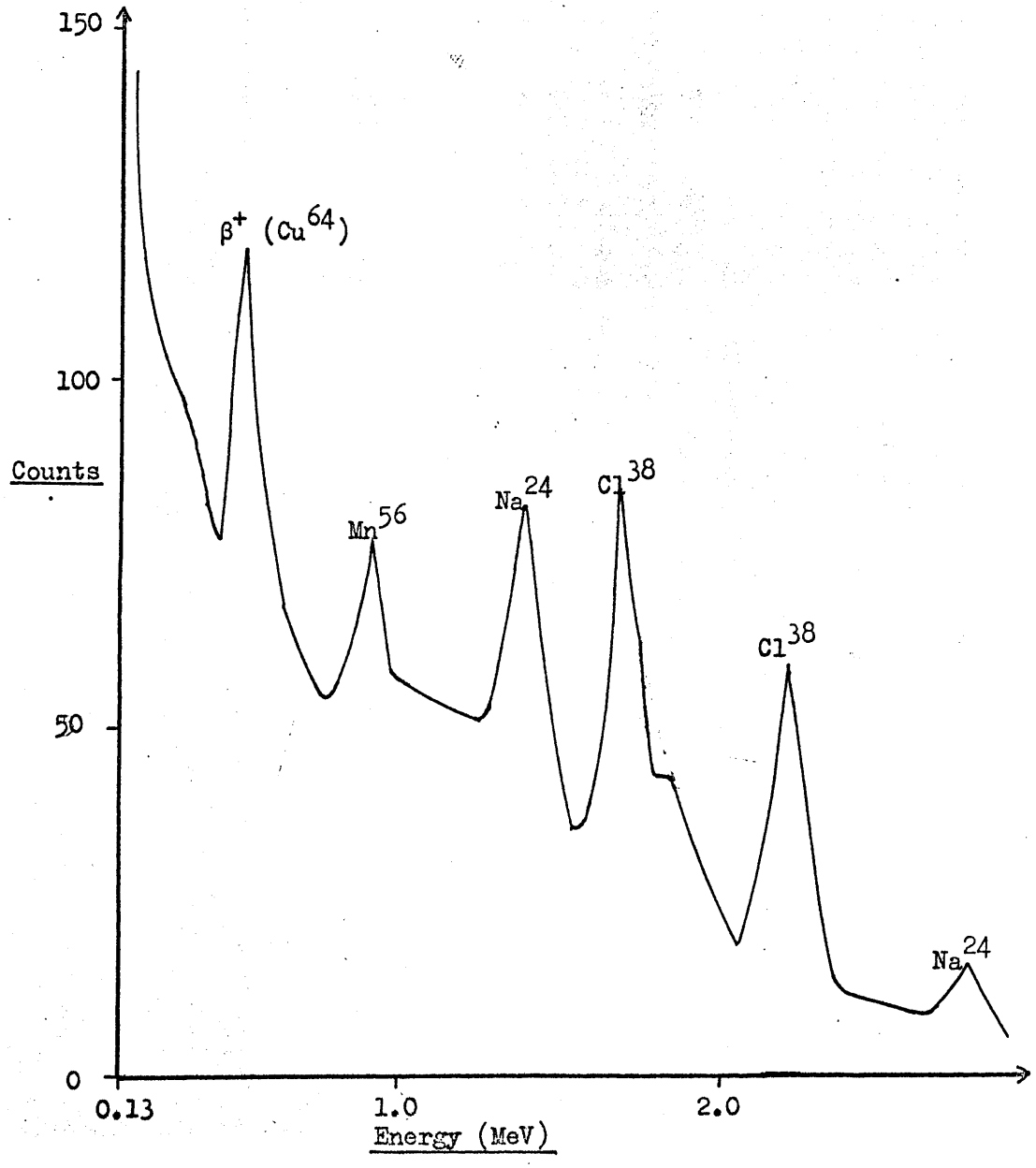


Figure C.4. Distribution of results for the Arsenic Content of Adrenal on Linear and Logarithmic Scales.

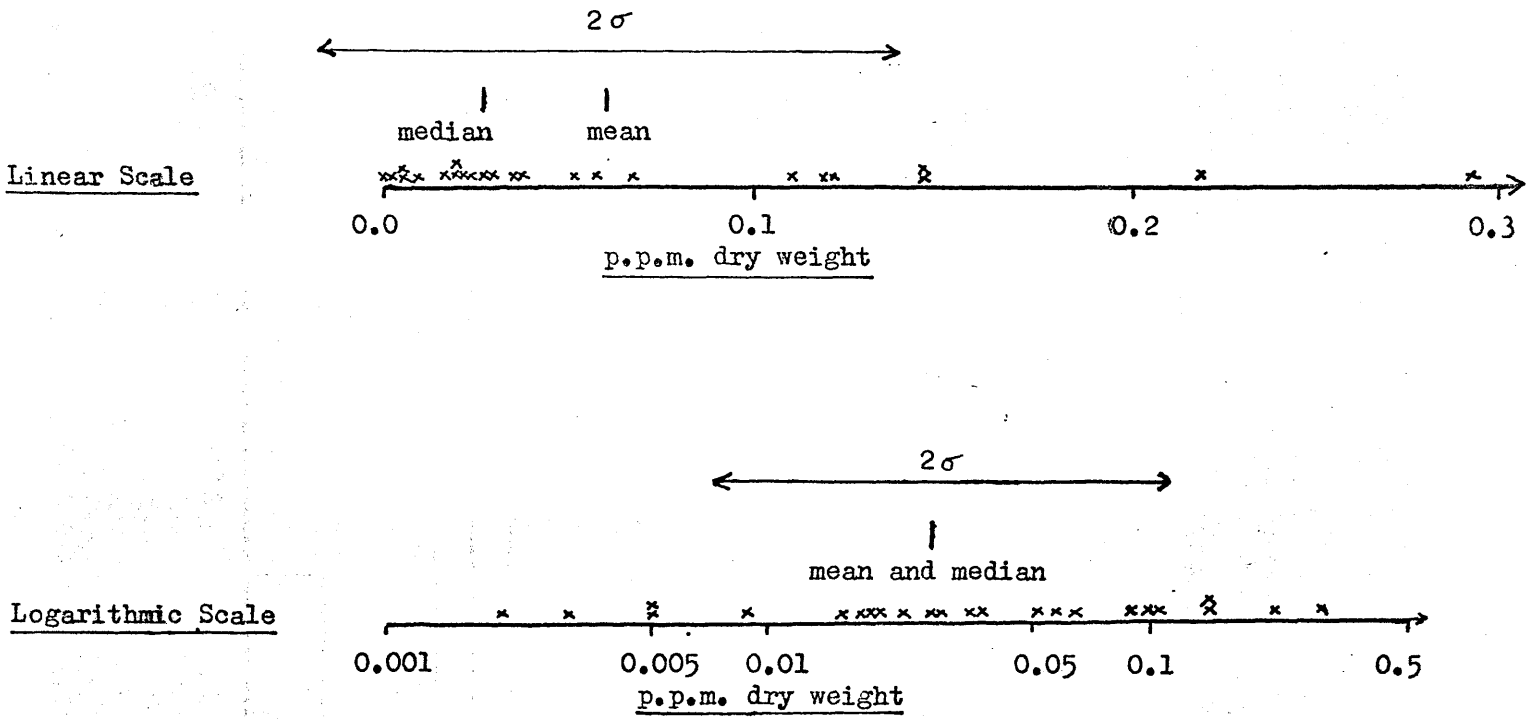


Figure C.5. Sectional Analysis of Hair from Stirk.